THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

SPECIAL MEETING

A SPECIAL Meeting of the Society was held at 11 a.m. on Tuesday, September 20th, 1960, in the Lecture Theatre, The Royal Institution, 21 Albemarle Street, London, W.1. The Chair was taken by Mr. J. G. Sherratt, B.Sc., F.R.I.C., P.A.I.W.E., Vice-President of the Society.

The subject of the meeting was "The Chemist and Food Quality" and the following papers were presented and discussed: "The Food Analyst To-day and Yesterday," by A. J. Amos, B.Sc., Ph.D., F.R.I.C.; "Some Applications of Research to the Study and Control of Consistency in Certain Foods," by E. H. Steiner, B.Sc., F.R.I.C.; "Estimation of the Polyphenolic Oxidation Products in Tea as an Assessment of Tea Quality—the Spectrophotometric Estimation of Theaflavins and Thearubigins in Black Tea Liquors," by E. A. H. Roberts, M.A., D.Phil., and R. F. Smith, B.Sc., F.R.I.C.; "The Analysis of Volatile Strawberry Flavours," by D. S. Bidmead, A.R.I.C.

ORDINARY MEETING

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, October 5th, 1960, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Mr. R. C. Chirnside, F.R.I.C.

The following papers were presented and discussed: "Paper Chromatography of Some Organo-tin Compounds," by D. J. Williams, B.Sc., and J. W. Price, Ph.D., F.R.I.C.; "A Procedure for Determining the Molar Extinction Coefficients of Metal Dithizonates," by H. M. N. H. Irving, M.A., D.Phil., D.Sc., F.R.I.C., and R. S. Ramakrishna, B.Sc.; "The Spectrophotometric Determination of Microgram Amounts of Calcium," by J. R. W. Kerr.

DEATHS

WE record with regret the deaths of

Harold Lowe
William Bayliss Shaw.

The Separation and Determination of the Platinum Metals

By S. T. PAYNE

(*The Mond Nickel Company Ltd., The Refinery, Bashley Road, London, N.W.*10)

The sample is attacked by a peroxide fusion; osmium and ruthenium are distilled as the volatile tetroxides and subsequently determined absorptiometrically or gravimetrically. The remaining platinum metals are complexed with nitrite and the base metals removed by a combination of hydrolysis and ion exchange. After destruction of the nitrite complexes with perchloric acid, the platinum metals are separated by cellulose chromatography on a single column in two stages. The fractions are treated individually to produce solutions from which the metals may be determined absorptiometrically or gravimetrically according to the amounts present.

An account is given of the experimental work leading to the development of the method, together with results for the separation of prepared mixtures.

For many years, the analysis of materials containing the platinum metals has usually involved, at some stage, a smelting operation to concentrate them in a lead button. Subsequent recovery involved scorification and then parting with nitric acid, which dissolved most of the palladium and left the remainder of the precious metals as an insoluble residue.

A hot sulphuric acid attack on the insoluble portion extracted rhodium together with small amounts of the other members of the group. Recovery and determination of the remaining metals was usually a lengthy and tedious process, almost always resulting in incomplete separations that had to be repeated until the pure metals were obtained.

Of all the platinum metal separations, that of rhodium from iridium is perhaps the most difficult, few really selective reagents having been proposed to date.[1]

The concentration or recovery of the precious metals from low-grade materials is not dealt with here, but a rapid and accurate method is described for separating and determining the individuals from a rich mixture or concentrate, which may also contain associated base metals, such as nickel, copper, bismuth and tin.

The operations involve preliminary distillation of ruthenium and osmium and then separation of the remaining platinum metals as a group from certain base metals by the well known nitrite reaction. Ion exchange and chromatography on cellulose form the basis of the main separations; spectrophotometric or gravimetric methods are used for the final determinations.

Distillation of ruthenium and osmium as the volatile tetroxides is a well established procedure and is conducted either by passing chlorine through an alkaline solution of the ruthenate or osmate or by oxidation with bromate from a weakly acid solution. The latter method has been described in some detail by Schoeller and Powell[2] and is the preferred technique.

Some papers[3,4,5] have been published on the separation of the platinum metals by ion exchange, but in the proposed method it is used for separating them as a group from large amounts of base metals, particularly sodium and nickel, which are present as the result of a peroxide fusion of the sample in a nickel crucible.

The chromtographic separation is based on the work of Rees-Evans, Ryan and Wells,[6] but has been modified to permit platinum, palladium, rhodium and iridium to be separated on a single column without the need for a second operation to part iridium and platinum.

The spectrophotometric method used for the final determination of platinum is a modification of the well known stannous chloride reaction, and those for rhodium and palladium depend on the colours of their chloro complexes under suitably standardised conditions. A method is also proposed for the spectrophotometric determination of small amounts of iridium by measuring the colour of its quadrivalent form under strictly controlled conditions.

At certain stages of the procedure it is necessary to use perchloric acid, and it should be emphasised that the conditions of its use must be strictly followed if accidents are to be avoided. It is also incumbent upon the operator to ensure that adequate means for disposal of perchloric acid fumes are available.

Experimental

Solution of the sample and removal of osmium and ruthenium—

Most materials rich in platinum metals are attacked by fusion with sodium peroxide. As there is usually a considerable amount of silica present, it should be removed by preliminary treatment with hydrofluoric acid, preferably in the nickel crucible subsequently to be used for the peroxide fusion. Nickel is substantially resistant to attack by hydrofluoric acid.

The silica-free residue was fused with sodium peroxide, the melt extracted with water, and the extract transferred to a distillation flask. Osmium and ruthenium were then removed by distillation as described by Schoeller and Powell.[2]

Separation of base metals by hydrolytic precipitation—

The solution in the flask after distillation of ruthenium and osmium usually contained tin, bismuth, lead, nickel, etc., together with large amounts of sodium salts. It was originally intended to remove these by passing the re-acidified solution through a cation-exchange resin, but it was found that some of the platinum metals, particularly palladium, were retained together with the base metals. When, however, the platinum metals were first complexed with nitrite, complete recovery was obtained.

The nitrite treatment precipitated the hydrolysable base metals, and the precipitate had to be removed before passing the solution through an ion-exchange column. To avoid the necessity of washing the frequently bulky precipitate, the unfiltered solution was adjusted to a suitable volume and half this volume of filtrate was collected. This procedure assumed that there was a homogeneous distribution of the platinum metals between the solution and precipitate, and in practice this was very nearly so. For the highest accuracy, or when silver was present in the original material, a re-treatment of the precipitate in the same manner ensured a proportional recovery without the need for much washing of the precipitate at any stage.

Removal of soluble cations by ion exchange—

The nitrited solution after filtration still contained large amounts of sodium salts and nickel, etc.; these were removed by passing the solution through a column of Zeo-Karb 225. Early attempts with this procedure were unsuccessful owing to the disruption of the column by the liberation of free nitrous acid, and it was obvious that excess of nitrite would have to be removed if the ion-exchange stage was to be satisfactory. On the other hand, too vigorous removal of nitrate resulted in partial break-down of the nitro complexes of the platinum metals, with consequent retention of palladium on the column. These difficulties were overcome by making the solution 1 per cent. in acid and boiling for not more than 1 minute. Subsequent removal of the cations was then satisfactory, and tests showed that no platinum metals were retained by the resin.

Chromatographic separation of the platinum metals—

The chromatographic separation of platinum, palladium, iridium and rhodium was first attempted by Rees-Evans, Ryan and Wells's method,[6] in which the metals must be in the form of their chloro acids. The nitrite complexes of the platinum metals, particularly those of iridium and rhodium, are extremely stable, and attempts to re-convert them to the required form by evaporation and boiling with hydrochloric acid proved unsuccessful. Always there remained small amounts of the undecomposed iridium and rhodium complexes, even after evaporation to dryness, which resulted in poor separations. It was found, however, that evaporation with perchloric acid completely decomposed the nitro and nitrosyl complexes of all the platinum metals. In this treatment, the excess of perchloric acid must be fumed away, and, unless a certain amount of alkali salt was present, partial reduction to metal occurred. Rees-Evans, Ryan and Wells suggested the use of zinc chloride to prevent reduction of the chloro complexes, as the presence of sodium chloride was found to be detrimental in the subsequent chromatographic separation. This was not successful in the proposed method, as, owing to the formation of zinc perchlorate, perchloric acid was liberated on re-conversion to the chloro acid, and separation was again poor. The difficulty was finally overcome by the use of lithium chloride, as the required stoicheiometric amount of this salt

was small. Further, lithium chloride is soluble in the ketone used for the separation and is therefore quickly removed from the system.

In the method proposed by Rees-Evans, Ryan and Wells, platinum and iridium were eluted together, with palladium following as a separate fraction and rhodium remaining on the column. Elution was conducted under oxidising conditions with *iso*butyl methyl ketone containing chlorate as the oxidising agent. Subsequently, the iridium and platinum fraction was chromatographed again, this time under reducing conditions with stannous chloride to retain iridium at the top of the column while platinum passed through. Recovery of platinum then required prior volatilisation of the tin as stannic chloride, by oxidation with bromine, etc. and distillation.

In attempts to speed up the process, it was realised that, if a satisfactory reducing agent that did not affect the other platinum metals could be found for iridium, it should be possible to extract first the platinum and then the palladium as before, leaving iridium with the rhodium at the top of the column. Subsequent elution under oxidising conditions would then remove iridium, leaving only the rhodium. Hence all four elements could be separated in two operations on a single column.

Earlier work in this laboratory had led to the use of hydroquinone for reducing iridium to the tervalent state, and its use fulfilled the requirement of reducing iridium in the cold without effect on the other platinum metals. It is soluble in *iso*butyl methyl ketone and easily destroyed in subsequent operations.

In separations by this combined procedure it was observed that, with certain proportions of platinum and palladium, *e.g.*, when one was greatly in excess of the other, incomplete separations were obtained, although partition on the column appeared to be perfectly satisfactory. Usually, under these extreme conditions, the palladium retained a small amount of platinum in the bivalent state.

Mostly, however, it was a simple matter to treat both the palladium and platinum fractions with dimethylglyoxime and to combine the filtrates for recovery of platinum and the precipitates for recovery of palladium; this course was adopted in the final method.

Treatment of the individual fractions—

Evaporation of the first fractions to remove the ketone proved a somewhat hazardous operation. Traces of residual perchloric acid extracted in the early stages found their way into the platinum fraction, and to a lesser extent into the palladium fraction, and caused severe polymerisation of the ketone with the formation of black resinous substances. Apart from the difficulty of completing the evaporation in the presence of these products, spontaneous explosions usually occurred with consequent loss of the sample. Attempts to overcome these undesirable characteristics led to the use of lithium carbonate for neutralising the acid present in the ketone. Addition of an excess of this reagent before the evaporation was begun resulted in a clean residue, substantially free from obnoxious organic matter. The presence of lithium salts was of no consequence during subsequent operations.

After removing the ketone and treating the residues with nitric and perchloric acids, re-conversion to the chloro acids was carried out by boiling with hydrochloric acid.

Determination of the individual platinum metals—

The methods used for determining individual platinum metals depended on the amount involved. If less than 10 mg were present, osmium, ruthenium and iridium were determined spectrophotometrically; amounts greater than this were determined gravimetrically. Any amount of platinum, palladium or rhodium was satisfactorily determined spectrophotometrically, but results were checked gravimetrically when required.

Osmium—The thiourea method for determining small amounts of osmium was quite satisfactory when applied directly to the solution obtained from the distillation. The pink colour developed rapidly at 75° C and was quite stable, but temperatures in excess of this were avoided to prevent the decomposition of thiourea with consequent precipitation of sulphur. The absorption curve for the complex is shown in Fig. 1; it can be seen that there is a suitable peak for measurement at 4800 A.

Larger amounts of osmium were determined gravimetrically by precipitation as hydrated osmium dioxide and subsequent reduction to metal under cover of hydrogen.

Iridium—Small amounts of iridium were determined spectrophotometrically by measuring the difference in absorption between the oxidised and reduced forms of the chloro acid.

Early attempts at a method based on this procedure failed, since it was difficult to ensure the complete and reproducible oxidation of this element.

Thus it was found that if a 5 per cent. iridium - platinum alloy was dissolved in various mixtures of hydrochloric and nitric acids and the solutions were then evaporated with hydrochloric acid several times, the resulting solutions after oxidation with chlorine water produced absorption curves with characteristics dependent upon the ratio of the two acids used for the dissolution. The curves for mixtures varying from (3 + 1) to (1 + 3) hydrochloric - nitric acid are shown in Fig. 2. The number of intermediate evaporations had no influence on the final shape of the curves and it was concluded that, during the dissolution process, nitro or nitrosyl iridium complexes were formed that were not subsequently broken down by these evaporations with hydrochloric acid.

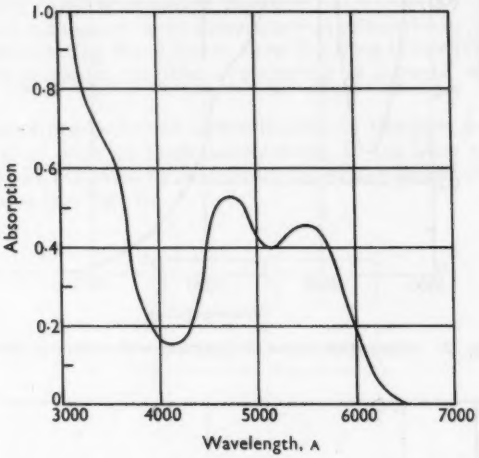

Fig. 1. Absorption curve of osmium - thiourea complex

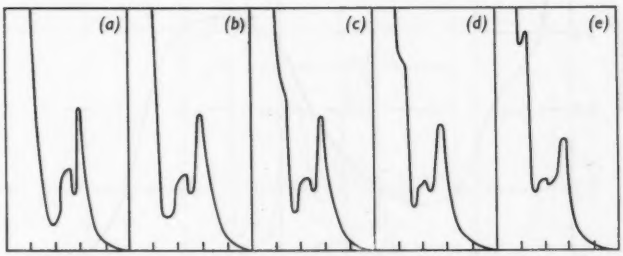

Fig. 2. Absorption curves for iridium in various hydrochloric - nitric acid mixtures: (*a*) 3+1; (*b*) 2+1; (*c*) 1+1; (*d*) 1+2; (*e*) 1+3

As perchloric acid was known to decompose nitrosyl and nitro complexes of iridium, vigorous treatment with this acid was tried, followed by re-conversion to the chloro acid and oxidation with chlorine water; reproducible results were then obtained. Further work showed that the chlorine water had to be freshly prepared and/or of consistent strength. As the latter condition was not easy to fulfil, the difficulty was overcome by the inclusion of a standard iridium solution treated with perchloric acid, etc., in the same way as for the assay.

Larger amounts of iridium were determined gravimetrically by precipitation as the hydrated dioxide from a perchloric acid solution with bromate as the oxidant. This method

gave an easily filterable product free from the usual peptisation troubles associated with this element. To prevent deflagration during ignition, paper and precipitate were washed with dilute ammonium chloride solution.

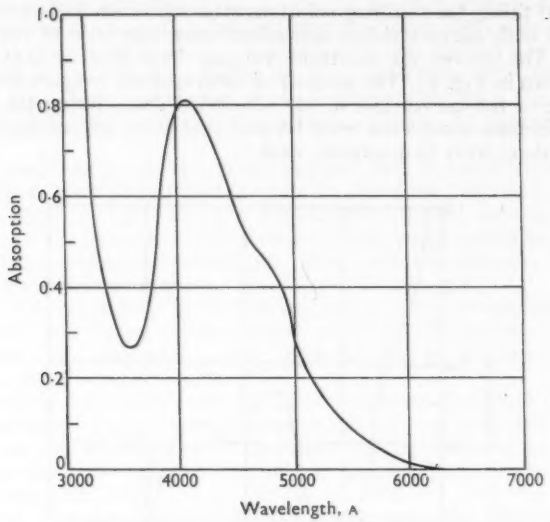

Fig. 3. Absorption curve of platinum with stannous chloride

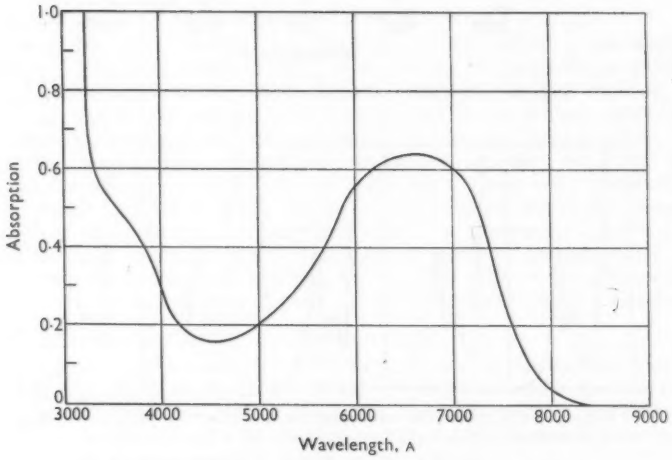

Fig. 4. Absorption curve of ruthenium - thiourea complex

Platinum—The well known stannous chloride reaction for platinum was successfully applied to determining this element after separation from other members of the group. The best reproducibility was obtained when the reaction was carried out in diluted hydrochloric acid (1 + 1); under these conditions the absorption curve exhibited a peak at 4030 A. At this wavelength and under the conditions described, the absorption was unaffected by variations in temperature between normal working limits. In the absence of interfering elements, however, the absorption at 4030 A is three times that at 3550 A (see Fig. 3) and

this fact can be used as a sensitive check on the completeness of the separation, since the presence of any interfering element (including any undesirable base metals) causes a disproportionately high reading at 3550 A with consequent alteration of the ratio.

Ruthenium—Small amounts of ruthenium were determined by the well known thiourea reaction, but, when the method was applied to the solution from a bromate distillation, results were unreliable owing to the presence of bromide. This difficulty was overcome by evaporating the solution to fumes with sulphuric acid to remove all halogens.

The presence of sulphuric acid was unimportant, but it was necessary to re-convert the ruthenium to the chloro complex by boiling with dilute hydrochloric acid before applying the test. The colour was developed in dilute hydrochloric acid (1 + 2), with heating to speed the reaction, but sulphur was precipitated if the solution was heated at too high a temperature for an excessive time; heating at 50° C for 30 minutes gave entirely satisfactory results. The absorption curve is shown in Fig. 4.

Larger amounts of ruthenium were determined gravimetrically, excellent results being obtained merely by evaporating the solution from the bromate distillation to small volume, transferring to a tared porcelain crucible, evaporating to dryness, and igniting the residue in hydrogen.

Rhodium—The spectrophotometric determination of rhodium has been the subject of a considerable amount of work in these laboratories; it has been established that a high degree of accuracy may be obtained by measuring the absorption of the chloro acid at 5150 A under suitable conditions (see Fig. 5).

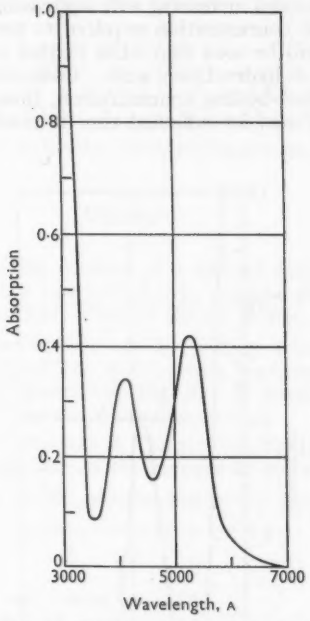

Fig. 5. Absorption curve of hexachlororhodic^{III} acid

The absorption of the chloro acid varies considerably with time according to the concentration of free hydrochloric acid in the solution. This may be due to hydrolysis, *i.e.*, replacement of Cl by OH groups to form intermediates of the type $H_3[RhCl_5OH]$, $H_3[RhCl_4(OH)_2]$, etc., to an equilibrium (according to the amount of free acid) between $H_3[RhCl_6]$ and a form of rhodium chloride in which the rhodium exists in the cationic state. In support of the latter is the fact that a certain amount of rhodium is usually retained by

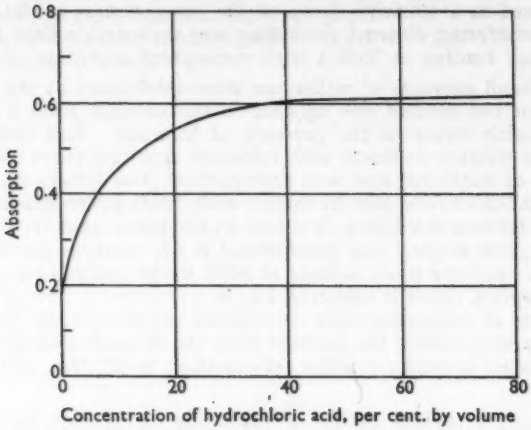

Fig. 6. Absorption curve of hexachlororhodic^{III} acid in various concentrations of hydrochloric acid

a cation-exchange resin, the amount increasing with decreasing acid concentration. Attempts to discover the minimum acid concentration required to prevent instability resulted in the graph shown in Fig. 6. It will be seen that little further increase in absorption at 5150 A occurs beyond 50 per cent. v/v hydrochloric acid. Conveniently, diluted hydrochloric acid (1 + 1) is very near to constant-boiling concentration, thus it is necessary only to boil the solution with a large excess of acid for sufficient time to reach equilibrium.

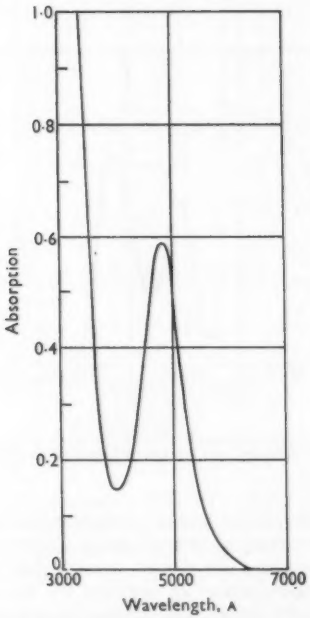

Fig. 7. Absorption curve of tetrachloropalladic^{II} acid

Palladium—Palladium was determined spectrophotometrically as the chloro acid, the absorption curve of which exhibits a peak at 4700 A (see Fig. 7). To be certain that conditions were reproducible, boiling with diluted hydrochloric acid (1 + 1) was carried out as with the rhodium. There was a marked temperature coefficient, but, as a standard was usually run at the same time as the assay, this was of no consequence, provided the solutions were set aside for a short time before measuring the absorption.

GRAVIMETRIC RECOVERY OF THE INDIVIDUAL METALS—

Apart from the methods already described, all the fractions may be treated for gravimetric recovery of the individual metals if required; suitable procedures are described below.

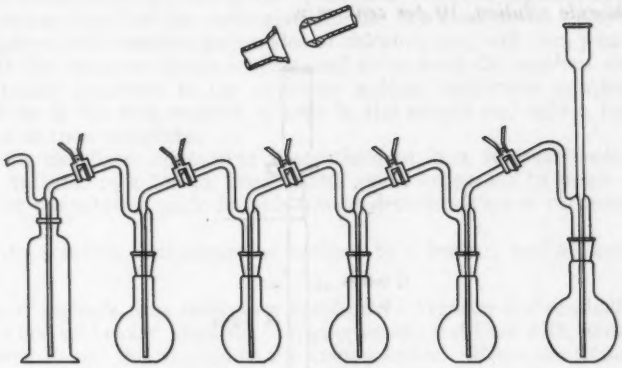

Fig. 8. Ruthenium distillation apparatus

METHOD

APPARATUS—

Ruthenium distillation train—This consists of a 500-ml distillation flask with dropping funnel, four 250-ml receivers and a Drechsel bottle. Connections are made via Quickfit & Quartz spherical joints; the complete train is shown in Fig. 8.

Ion-exchange column—The column is made by joining a length of Pyrex-glass tubing (14 inches × 1¼ inches) to the cut-off top and bottom portions of a standard Quickfit & Quartz CR/32/20 chromatograph column (see Fig. 9). It is filled with Zeo-Karb 225 and prepared by acid washing, etc., in the usual manner.

Cellulose column—A standard Quickfit & Quartz CR/32/40 column equipped with a reservoir and tap adapter. Preparation of the column is described below.

Spectrophotometer—A Unicam SP500 spectrophotometer and 40-mm cells.

REAGENTS—

Sodium peroxide, granules.
Hydrochloric acid, sp.gr. 1·18, *and diluted* (1 + 1).
Sodium bromate solution, 10 *per cent. w/v.*
Ethanolic sodium hydroxide solution—A 10 per cent. solution of sodium hydroxide in 10 per cent. industrial ethanol.
Sulphuric acid, diluted (1 + 1)—Analytical-reagent grade.
Nitric acid, sp.gr. 1·42.
Sodium nitrite—Analytical-reagent grade.
Perchloric acid, 60 *per cent.*—Analytical-reagent grade.
Hydroquinone, pure, and a 5 *per cent. w/v solution.*
Thiourea, pure.
Dimethylglyoxime solution, sodium salt, 5 *per cent. w/v.*

Sodium hydroxide, pellets—Analytical-reagent grade.
Sodium hydrogen carbonate, pure.
iso*Butyl methyl ketone.*
Acid solvent—*iso*Butyl methyl ketone *plus* 2 per cent. v/v of hydrochloric acid.
Reducing solvent—Acid solvent *plus* 0·05 per cent. of hydroquinone.
Oxidising solvent—Freshly prepared (see p. 709).
Sodium chlorate, pure, and a 2 per cent. w/v solution.
Cellulose powder, Whatman standard grade.
Hydrobromic acid—Analytical-reagent grade.
Stannous chloride solution, 20 per cent. w/v in diluted hydrochloric acid (1 + 4).
Chlorine water—Freshly prepared as required.
Lithium carbonate, pure.
Lithium chloride solution, 10 per cent. w/v.

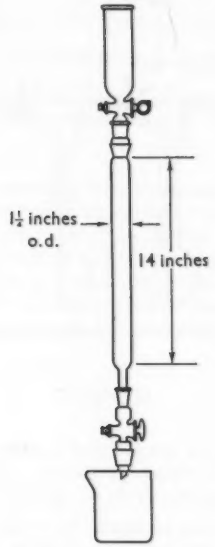

Fig. 9. Ion-exchange apparatus

PROCEDURE—

Dissolution of sample—Weigh the crushed sample (containing not more than 250 mg of platinum metals) into a nickel crucible, and moisten with a few drops of water. Introduce about 5 ml of hydrofluoric acid, and evaporate the mixture to dryness. Heat the crucible gently, and raise the temperature slowly to bright red heat (under cover of hydrogen if osmium is to be determined), taking care to avoid loss by spitting.

When cool, add 10 g of sodium peroxide, and mix the sample intimately with the aid of a glass rod. Heat the crucible slowly until the contents sinter, maintain in this state for 30 minutes, and then raise the temperature to bright red heat, swirling the melt to ensure complete attack. Avoid prolonged heating at this temperature to minimise attack on the crucible; 1 or 2 minutes is usually sufficient.

Set the crucible aside to cool, and then place it in a 400-ml beaker, and just cover with water. When the violent reaction has subsided, rinse the cover and sides of the beaker, remove the crucible with a pair of forceps, wash it inside and out, clean the surface with a rubber-tipped glass rod, and wash again.

Transfer the contents of the beaker, with washings, to the ruthenium distillation flask, and connect the flask to the train. Place 150 ml of diluted hydrochloric acid (1 + 1) in each

receiver and 150 ml of ethanolic sodium hydroxide solution in the Drechsel bottle. Meanwhile, place 20 ml of hydrochloric acid in the nickel crucible, and heat to dissolve any particles adhering to the surface. With a suction pump, draw a slow current of air through the distillation train, and transfer the acid to the distillation flask. Rinse the crucible with a further 15 ml of acid, and add the rinsings to the contents of the flask.

Distillation of osmium and ruthenium—Heat the flask slowly, and boil the contents gently for about 10 minutes, maintaining a steady flow of air through the train. Add 10 ml of 20 per cent. w/v sodium chlorate solution a few drops at a time, and then 35 ml of 10 per cent. w/v sodium bromate in 5-ml portions at intervals of about 2 minutes. Boil the solution continuously while the additions are made and then for a further 30 minutes to complete the volatilisation of osmium and ruthenium.

Some 15 minutes before the end of the distillation, heat the contents of the first receiver to boiling to reduce most of the ruthenium to the tervalent state. Any ruthenium still unreduced, together with osmium and oxides of chlorine, etc., will then pass into the second receiver. Heat the contents of this in turn and so on until the osmium and other volatile products are finally absorbed in the ethanolic sodium hydroxide solution. Most of the ruthenium will be in the first receiver, a little in the second and only a trace in the third. The distillation is then complete.

Combine the distillates containing the ruthenium in a 1000-ml beaker, evaporate to small volume, transfer to a 150-ml beaker, and again evaporate to small volume, but not to dryness. Set the solution aside for subsequent determination of ruthenium, as described below.

Transfer the solution containing the osmium to a beaker, and set aside for the determination.

Treatment of raffinate from ruthenium distillation—Transfer the residual liquor from the distillation to a 600-ml beaker; rinse the dropping funnel and flask with 50 ml of hydrochloric acid, and cautiously add the rinsings to the main solution. Rinse the funnel and flask with hot water, cover the beaker, and boil the solution vigorously to reduce the volume to about 100 ml, taking care to remove the beaker before crystallisation of the salts causes severe bumping. Transfer the beaker to a low-heat hot-plate, and continue the evaporation until the contents are almost dry. On no account allow the salts to bake.

Nitrite separation—Dissolve the salts in about 150 ml of water, boil for 5 minutes, and then dilute to about 400 ml with hot water. To the boiling solution, add solid sodium nitrite a little at a time until the pH changes to 7 (measured with Johnson's test paper), and then, after another small addition of nitrite, boil for a further 5 minutes. When cool, transfer the solution and precipitate to a 500-ml calibrated flask, and make up to the mark with distilled water.

Mix the contents of the flask well, and filter through a Whatman No. 40 filter-paper, rejecting the first few millilitres of filtrate. Collect 250 ml of filtrate in a dry calibrated flask, and set aside; then continue the filtration, and allow the filter-paper and precipitate to drain. Wash the precipitate once with cold water, and discard the remaining filtrate.

Transfer the filter-paper and precipitate to a 400-ml beaker, add 2 ml of hydrochloric acid and 20 ml of water, and bring to the boil. Boil for 5 minutes, dilute to about 100 ml, and repeat the nitrite treatment as before. When cool, transfer the solution, precipitate and filter-paper to a 200-ml calibrated flask, and filter as before, but this time collect 100 ml of filtrate. Discard the remaining filtrate and precipitate. (Any gold in the original sample will be present in the precipitate.)

Transfer the two filtrates (which now represent one half of the original sample) to a 600-ml beaker, and rinse the flasks with distilled water. Add 4 ml of hydrochloric acid, and heat the solution to boiling. Boil for not more than 1 minute to remove excess of nitrous fumes, then cool as rapidly as possible, and adjust the pH to about 3 with sodium hydroxide.

Removal of base metals by ion exchange—The ion-exchange column is shown in Fig. 9. Regenerate the resin (Zeo-Karb 225) with diluted hydrochloric acid (1 + 1), and then elute with water until the effluent is neutral. Place the reservoir in position, and fill with the solution of nitrited platinum metals. Pass the solution through the column at the rate of about 1 drop per second, re-filling the reservoir as necessary. When all the solution has

been transferred, rinse the beaker two or three times, and add the washings likewise. Immediately before the last few millilitres of solution become absorbed, rinse the reservoir with about 20 ml of water, and allow this to pass through. Repeat with several successive 20-ml portions, fill the reservoir with water, and continue the elution until the effluent is neutral.

Treatment of ion-exchange effluent—Evaporate the effluent (now free from sodium, nickel, etc.) to small volume, and then transfer to a 250-ml beaker; add 2 ml of 10 per cent. w/v lithium chloride solution, and continue the evaporation to dryness. Moisten the residue with a few drops of water, add 10 ml of perchloric acid, and evaporate until copious fumes are evolved. Continue heating until all the free perchloric acid has been expelled, and then set aside to cool. Rinse the sides of the beaker with the minimum amount of water, and evaporate again until evolution of fumes ceases completely.

Treat the cooled residue with 5 ml of diluted hydrochloric acid (1 + 1), and evaporate to dryness. Repeat this treatment at least six times (up to ten times for materials very rich in iridium). Dissolve the residue in 20 ml of concentrated hydrochloric acid, and bring to the boil in a covered beaker. Continue boiling until the volume has been reduced to about 5 ml, remove the cover, and evaporate gently to about 2 ml.

It is most important not to allow the contents of the beaker to solidify at this stage, or the final treatment with 20 ml of hydrochloric acid will have to be repeated. If, however, the residual liquor tends to crystallise on cooling, add the absolute minimum of hydrochloric acid 1 drop at a time until solution is regained.

Chromatographic separation—The cellulose column should be freshly prepared as described below.

> Prepare a quantity of acid solvent by adding 20 ml of hydrochloric acid to 1 litre of *iso*butyl methyl ketone. Take 200 ml of this acid solvent, and add Whatman standard-grade cellulose powder until a thin slurry is formed. Pour the mixture into the column in the usual manner, adding sufficient to form a bed of cellulose about 35 cm deep. Allow to drain, leaving about 1 cm of solvent over the cellulose, and then set aside until required.

Add to the cold solution of platinum metals a small amount of solid hydroquinone and then 10 ml of reducing solvent. Stir vigorously until the iridium has been reduced, adding more solid hydroquinone if necessary.

Begin the column separation by opening the tap fully. Immediately the solvent has drained, introduce the first extract by careful decantation, avoiding transfer of any of the aqueous phase. Repeat the extraction with a further 10 ml of reducing solvent, and, as soon as the first extract has been absorbed, transfer in the same way. Continue the extractions in this manner, with 10 ml of reducing solvent at a time, and avoiding transfer of the aqueous phase. It is most important that the column should not be allowed to drain at any time to such an extent that air is introduced between the column wall and the cellulose.

As the elution proceeds the platinum band will move ahead, with the palladium band following somewhat more slowly. However, the platinum fraction should be collected as soon as the extractions are begun, as an almost invisible platinum band moves ahead of the main one and may otherwise be lost.

As soon as it is evident that all the palladium has been extracted and transferred to the column, make a final extract from the beaker with acid solvent not containing hydroquinone, then place the reservoir in position, fill with reducing solvent, and continue the elution once again. Meanwhile, set aside the beaker containing the iridium and rhodium.

When the main platinum band has reached the lower end of the column, a gap of some nches should have appeared between it and the following palladium band. Continue to collect the platinum fraction until just before the palladium starts to come through.

At this point, change the receiver, and collect the palladium fraction in the same way, replenishing the reservoir with reducing solvent as necessary. Immediately the last of the palladium comes through, remove the reservoir, and elute with two or three successive portions of acid solvent (non-reducing) to remove the hydroquinone and render the ensuing oxidation treatment fully effective. The column is then ready for the second stage, and meanwhile elution is stopped.

Iridium is extracted by elution with an oxidising solvent. Prepare this solvent freshly as described below.

Take 100 ml of acid solvent, and add 2 ml of hydrochloric acid and then 4 g of sodium chlorate. Stir thoroughly until the solution becomes cloudy, add 10 g of cellulose powder, and macerate. Decant the clear liquor into another beaker, and dilute 50 ml of this to 250 ml with acid solvent.

Extract iridium from the solution in the beaker in exactly the same way as for platinum and palladium, and use 10 ml of oxidising solvent each time. When all the iridium has been extracted and the dark-brown band is well clear of the residual pink rhodium band (despite care in avoiding transfer of the aqueous phase, a small amount of rhodium usually finds its way on to the column), place the reservoir in position, and continue eluting with acid solvent containing a little of the oxidising solvent. When it is certain that all the iridium has been collected, dilute the residual rhodium phase with a little water, remove the reservoir, and transfer the solution to the column. Rinse the beaker two or three times, and, when these washings have almost been absorbed, fill the reservoir with water and continue eluting. When the pink band has reached almost to the lower end of the cellulose, collect the rhodium fraction in a separate beaker.

Treatment of individual fractions—To the platinum and palladium fractions add 1 g of lithium carbonate for each 100 ml of ketone. To the iridium and rhodium fractions add 5 ml of 10 per cent. w/v lithium chloride solution. Evaporate the respective fractions by gentle boiling on an electric hot-plate in a well ventilated fume cupboard. Continue the evaporation to dryness, but avoid overheating the residue beyond the point necessary just to remove the ketone.

When cool, add 10 ml of water to each, and then 50 ml of nitric acid. Cover the beakers, and heat gently. When the first vigorous reaction has subsided, bring the solutions to the boil, and continue boiling until brown fumes are no longer evolved, adding more nitric acid if required. Then add 25 ml of perchloric acid, boil until most of the nitric acid has been expelled, remove the covers, and evaporate the solutions until fumes are evolved. Continue fuming until the volume has been reduced to about 5 ml for iridium or until the salts begin to crystallise for the other metals. Set aside the iridium fraction for subsequent determination (see p. 710). Add 50 ml of diluted hydrochloric acid (1 + 1) to the others; heat the solutions to boiling to re-convert the metals to the chloro complexes, and treat the rhodium and the platinum and palladium fractions as described below.

Rhodium fraction—Boil the rhodium fraction for at least 1 hour, adding more diluted hydrochloric acid (1 + 1) as necessary to maintain the volume. Finally, add 2 or 3 ml of chlorine water, boil for a further minute or so, cool, and accurately dilute to a suitable volume with diluted hydrochloric acid (1 + 1) for the absorptiometric determination. (Approximately 15 mg of rhodium per 100 ml is a suitable concentration.)

Platinum and palladium fractions—Evaporate the platinum and palladium fractions to about 20 ml, cool, and dilute to about 250 ml with cold water. Add a 5 per cent. solution of sodium dimethylglyoximate to each fraction to precipitate the palladium, and set aside for 30 minutes.

Filter the platinum fraction, containing little or no palladium, through a Whatman No. 40 filter-paper, rinse the beaker several times with cold water and the paper once or twice, filter the palladium fraction through the same paper, and combine the filtrates.

Place the paper and precipitate in a 400-ml beaker, add 20 ml of nitric acid and then 20 ml of perchloric acid, and heat the solution strongly until brown fumes are no longer evolved. Evaporate the solution until fumes of perchloric acid are evolved, and continue fuming until about 2 ml of acid remain. Finally, re-convert palladium to the chloride by boiling for a few minutes with diluted hydrochloric acid (1 + 1), and adjust to a suitable volume with the same acid (10 mg per 100 ml) for absorptiometric determination.

Evaporate the combined filtrates containing the platinum with nitric acid to destroy the excess of glyoxime, and, when the solution has been reduced to small volume, add an excess of hydrochloric acid, and boil until brown fumes are no longer evolved. Finally, make the solution up to a suitable volume for absorptiometric determination.

The procedure for the platinum and palladium fractions may be omitted when palladium is in excess of platinum, the respective solutions being adjusted to suitable volumes after the initial re-conversion to their chloro complexes.

DETERMINATION OF THE INDIVIDUAL METALS—

Osmium—If the amount of osmium present is more than about 10 mg, precipitate it as the hydrated dioxide, and determine it gravimetrically.[2]

Determine amounts of osmium less than 10 mg absorptiometrically as described below.

Boil the alkaline osmate solution from the distillation to expel most of the ethanol, cool, and make up to a suitable volume from which an aliquot representing 1 mg of osmium may be taken.

Transfer the aliquot to a 100-ml calibrated flask, neutralise with hydrochloric acid, keeping the solution as cool as possible, add 30 ml of concentrated hydrochloric acid and then 10 ml of 10 per cent. thiourea solution, and dilute to the mark. Mix well, immerse the flask in a water bath at 75° ± 2° C for 30 minutes, cool, and measure the absorption of the solution at 4800 A in a 40-mm cell.

Calculate the amount of osmium present by comparison with a standard prepared as described under "Standardisation," p. 712.

Iridium—Dilute the perchlorate solution containing the iridium to about 300 ml with hot water, and add sodium hydrogen carbonate solution until the pH changes to about 4. Add 10 ml of 10 per cent. sodium bromate solution, and boil gently for about 30 minutes to coagulate the precipitate, adding more sodium bromate solution if necessary to keep the pH between 6·5 and 7·0. Allow the precipitate to settle, and then filter through a Whatman No. 40 filter-paper, washing well with hot water. There is no tendency to peptisation with iridium precipitated in this manner, but a final wash with 5 per cent. ammonium chloride solution serves to prevent deflagration during the subsequent ignition.

Dry the paper and precipitate, ignite carefully, and treat the iridium dioxide with hydrofluoric and nitric acids in the usual manner. Collect the precipitate once again, ignite, reduce under hydrogen, and weigh as usual.

For small amounts of iridium (10 mg or less) an absorptiometric finish is probably more convenient. When this is so, adjust the perchlorate solution to a suitable volume from which an aliquot containing 1 mg of iridium may be taken.

Transfer two aliquots to 150-ml beakers, add 50 ml of diluted hydrochloric acid (1 + 1) to each, and boil for 5 minutes. When cool, transfer each solution to a 100-ml calibrated flask, make the first solution up to about 90 ml with diluted hydrochloric acid (1 + 1), and add 4 ml of freshly prepared chlorine water. Place the flask in a boiling-water bath for 30 minutes, and then cool, and adjust the volume with diluted hydrochloric acid (1 + 1). Meanwhile, treat the second solution with 2 drops of 5 per cent. hydroquinone solution, and dilute to the mark with diluted hydrochloric acid (1 + 1). With the reduced solution in the reference or water cell, measure the absorption of the oxidised solution at 4900 A in 40-mm cells. Calculate the amount of iridium by comparing the absorption with that of a standard prepared as described under "Standardisation," p. 712.

Platinum—Transfer an aliquot representing about 0·35 mg of platinum to a 150-ml beaker, add 5 ml of perchloric acid, and evaporate until fumes are evolved. While fumes are being evolved add dropwise about 10 drops of hydrobromic acid, and continue heating until most of the excess of perchloric acid has been removed; then cool, add 50 ml of diluted hydrochloric acid (1 + 1), and boil to re-convert the platinum to the chloro complex. Cool again, transfer the solution to a 100-ml calibrated flask, and dilute to about 80 ml with diluted hydrochloric acid (1 + 1). Finally, add 10 ml of 20 per cent. stannous chloride solution, and dilute to the mark. Mix well, set aside for 30 minutes, and then measure the absorption at 4030 A in a 40-mm cell; compare with a standard prepared as described under "Standardisation," p. 712.

If a gravimetric finish is preferred, dilute the original perchlorate solution resulting from the ketone extraction (or an aliquot therefrom) to a suitable volume, and precipitate the platinum as metal by reduction with magnesium in the usual manner. Treat the ignited precipitate with hydrofluoric acid, etc., to remove silica, re-ignite, and weigh as metal.

Ruthenium—If more than 10 mg of ruthenium are present, carry out the determination gravimetrically as described below.

Transfer the previously evaporated solution to a tared porcelain crucible, and evaporate to dryness in a water bath. When dry, place the crucible on a hot-plate, and gradually increase the temperature to full heat during a period of about 30 minutes.

Ignite over a burner, slowly at first then at full heat, maintaining a cover of hydrogen throughout. Remove the source of heat, and allow the crucible to cool under hydrogen, reducing the hydrogen pressure gradually until, finally, the crucible can be removed and left to cool completely in air.

Brush the loose residue into a platinum dish, and treat with hydrofluoric acid. Evaporate to dryness, extract the residue with dilute nitric acid, filter, and wash with hot water, replacing the paper and precipitate in the original porcelain crucible. Dry, ignite, and, finally, reduce with hydrogen in the usual manner; re-weigh the crucible and contents, and determine the weight of metal by difference.

For less than 10 mg of ruthenium, carry out the determination spectrophotometrically, as described below.

Dilute the original solution to 100 ml in a calibrated flask, and extract an aliquot equivalent to about 1 mg of ruthenium. Transfer the aliquot to a 150-ml beaker, add 5 ml of diluted sulphuric acid (1 + 1), and evaporate until copious fumes are evolved. After fuming for 2 or 3 minutes, cool the solution, add 60 ml of diluted hydrochloric acid (1 + 1), and boil for 5 minutes.

Cool the solution, and transfer to a 100-ml calibrated flask, rinsing the beaker with water to bring the volume to about 85 ml. Add 10 ml of 10 per cent. thiourea solution, mix well, and heat the flask in a water-bath at 50° ± 2° C for 30 minutes. Cool, make up to the mark with water, and measure the absorption at 6750 A in a 40-mm cell. Calculate the amount of ruthenium present by comparison with a standard prepared as described under "Standardisation," p. 712.

Rhodium—The rhodium solution may contain a trace of iridium if the original material contained a high percentage of this metal; it is necessary to determine it to correct the main iridium figure.

Transfer a small portion of the previously oxidised and diluted solution (see "Treatment of Individual Fractions," p. 709) to a dry beaker, and add a few crystals of hydroquinone. With this reduced solution in the reference or water cell, measure the difference in the absorption of the oxidised solution at 4900 A in 40-mm cells. Calculate the amount of iridium present by reference to the iridium standard, and correct the main iridium figure as necessary. Then filter the reduced solution through a dry Whatman No. 540 filter-paper into a dry beaker, and measure the absorption of this against water at 5150 A in 40-mm cells. Calculate the amount of rhodium present by comparison with a standard prepared as described under "Standardisation," p. 712.

NOTE—It is essential not to filter the oxidised solution before determining the iridium content, as some iridium may be reduced by the filter-paper.

Palladium—Measure an aliquot representing about 10 mg of palladium, and make up to 100 ml in a calibrated flask with diluted hydrochloric acid (1 + 1). Mix well, filter a portion through a dry Whatman No. 540 filter-paper into a dry beaker, and measure the absorption at 4700 A in 40-mm cells. Calculate the amount of palladium present by comparison with a standard prepared as described under "Standardisation," p. 712.

Large amounts of palladium can, if desired, be determined gravimetrically by carefully igniting the original glyoxime precipitate under hydrogen. If this procedure is adopted, the precipitate must be thoroughly washed with cold water to remove lithium salts, etc.

STANDARD SOLUTIONS—

Osmium—Distil some osmium from any readily obtainable salt by the method described on p. 707, and standardise a portion of the distillate gravimetrically, also as previously described. Dilute the distillate to produce a solution containing 1 mg of osmium per ml.

Iridium—Weigh 1·1 g of pure iridium sponge into a nickel crucible, and add 10 g of sodium peroxide. Mix the contents intimately, and heat gently until the mass sinters. Maintain at this temperature for 30 minutes, and then raise the temperature to bright red heat for about 2 minutes. Set aside to cool, extract the melt with cold water, transfer to a 500-ml beaker, and acidify with hydrochloric acid. Evaporate the solution to small volume, dilute the salts to about 300 ml, and filter. Pass the solution through a column of Zeo-Karb 225 to remove sodium and nickel, etc., and then dilute the effluent to 1000 ml with 1 per cent. hydrochloric acid.

Standardise the solution as described below.

By pipette, place 50 ml of the solution in a 500-ml beaker, add 10 ml of nitric acid and then 10 ml of perchloric acid, and evaporate until only about 2 ml of acid remain. Dilute to 250 ml with hot water, and precipitate the iridium with sodium bromate as described under "Iridium," p. 710.

Platinum—Weigh 0·1 g of pure platinum sponge, and dissolve in aqua regia. Evaporate the solution several times with hydrochloric acid, and, finally, dilute to 1000 ml with diluted hydrochloric acid (1 + 1).

Ruthenium—Dissolve commercial ruthenium chloride in 1 per cent. hydrochloric acid, and dilute to give a solution containing about 1 per cent. of ruthenium. Evaporate 10 ml of this solution in a tared porcelain crucible, and determine the ruthenium gravimetrically as described under "Ruthenium," p. 710. On the basis of the assay, further dilute the solution to produce a final standard solution containing 1 mg of ruthenium per ml.

Rhodium—Weigh 5·5 g of pure rhodium sponge into a 250-ml conical flask, and add 50 ml of concentrated sulphuric acid and then 10 g of sodium sulphate. Boil gently in a covered flask until all the rhodium has been attacked. Cool, and cautiously dilute the solution to about 200 ml; then filter, and dilute to about 800 ml.

Precipitate the rhodium as hydroxide by careful addition of sodium hydroxide solution to the nearly boiling solution until the pH changes to 7 (measured with a Johnson's test paper).

Collect the rhodium hydroxide on a Buchner funnel, and wash well with hot water. Transfer the precipitate to the original beaker, re-dissolve in an excess of hydrochloric acid, and evaporate to small volume. Add 250 ml of diluted hydrochloric acid (1 + 1), and boil for 1 hour, adding more hydrochloric acid if necessary to maintain the volume. Finally, cool, filter through a Whatman No. 540 filter-paper, and make up to 500 ml with diluted hydrochloric acid (1 + 1).

Standardise the solution gravimetrically by taking an aliquot and reducing the rhodium to metal with magnesium in the conventional manner.

Palladium—Weigh 1·0 g of pure palladium sponge, and dissolve in aqua regia. When solution is complete, evaporate several times with hydrochloric acid, and dilute to 1000 ml with diluted hydrochloric acid (1 + 1).

STANDARDISATION—

Osmium—Transfer 1 ml of standard osmium solution to a 100-ml calibrated flask, add 60 ml of diluted hydrochloric acid (1 + 1) and then 10 ml of 10 per cent. thiourea solution, and dilute to the mark with water. Mix well, and continue as described under "Osmium," p. 710.

Iridium—Transfer 1 ml of standard solution to a 150-ml beaker. Add 1 ml of nitric acid and then 5 ml of perchloric acid, and evaporate until only about 2 ml remain, taking care to avoid evaporating to dryness. Cool, add 50 ml of diluted hydrochloric acid (1 + 1), and boil for 5 minutes. Transfer the solution to a 100-ml calibrated flask, and continue as described under "Iridium," p. 710.

NOTE—It is essential that the same batch of chlorine water is used for the assay as for the standard.

Platinum—Transfer 5 ml of standard solution to a 100-ml calibrated flask, dilute to about 80 ml with diluted hydrochloric acid (1 + 1), add 10 ml of 20 per cent. stannous chloride solution, and continue as described under "Platinum," p. 710.

Ruthenium—By pipette, place 1 ml of standard solution in a 150-ml beaker, add 5 ml of diluted sulphuric acid (1 + 1), evaporate the solution until fumes are evolved, and continue as described under "Ruthenium," p. 710.

Rhodium—Take 20 ml of standard solution, transfer to a 250-ml beaker, and add 50 ml of diluted hydrochloric acid (1 + 1). Boil the solution gently for 15 minutes, and then cool, and make up to 100 ml with diluted hydrochloric acid (1 + 1). Measure the absorption at the same time as that of the assay at 5150 A in 40-mm cells.

Palladium—Take 10 ml of standard solution and transfer to a 250-ml beaker. Add 5 ml of nitric acid and then 5 ml of perchloric acid, and evaporate the solution until about 2 ml of acid remain, taking care to avoid evaporating to dryness. Set aside to cool, then

add 50 ml of diluted hydrochloric acid (1 + 1), and boil for 10 minutes. Cool again, and dilute the solution to 100 ml in a calibrated flask with diluted hydrochloric acid (1 + 1). Measure the absorption at the same time as that of the assay at 4700 A in 40-mm cells.

Results

Synthetic mixtures were prepared in two groups—

(*i*) those containing osmium and ruthenium;

(*ii*) those containing iridium, platinum, rhodium and palladium.

The mixtures were analysed by the proposed methods; the results are shown in Tables I and II.

Table I

Determination of osmium and ruthenium

Method	Osmium added, mg	Osmium found, mg	Ruthenium added, mg	Ruthenium found, mg
Absorptiometric	2·5	2·4	2·5	2·5
	5·0	4·8	5·0	5·1
	10·0	9·9	10·0	9·9
Gravimetric	25·0	25·5	25·0	25·1
	50·0	49·2	50·0	50·3
	100·0	98·2	100·0	100·1

Table II

Determination of iridium, platinum, rhodium and palladium

	Iridium	Platinum	Rhodium	Palladium	Totals
Added, mg	45·0	30·0	100·0	10·0	185·0
Found, mg	44·9*	30·0	99·9	9·9	184·7
Added, mg	8·0	120·0	25·0	60·0	213·0
Found, mg	7·9	121·0*	25·0	59·8	213·7
Added, mg	108·0	75·0	10·0	20·0	213·0
Found, mg	108·2*	75·1	10·0	20·2	213·5
Added, mg	25·0	7·6	150·0	50·0	232·6
Found, mg	25·1*	7·6	150·6	49·8	233·1
Added, mg	74·0	20·0	72·0	102·0	268·0
Found, mg	73·8*	20·4	72·1	101·8	268·1

* Determined gravimetrically; all other results obtained absorptiometrically.

Several concentrates containing a wide variety of base metals have also been analysed over a period of many months, and the results have shown good agreement with those obtained by established techniques.

Conclusions

A combination of modern techniques for the analysis of mixtures of the platinum metals has been described. The method gives good results with a wide variety of products, including complicated concentrates containing several undesirable base metals.

The time taken for a complete analysis has been reduced to little more than one week, the accuracy comparing favourably with the older classical techniques. The frequent use of perchloric acid calls for some care, and it is essential that a properly designed fume-extraction system is employed, with a separate flue for ketone evaporations.

Addendum

Recent work has shown that a more rapid chromatographic separation can be achieved by incorporating a proportion of tri-*n*-butyl phosphate with the ketone. The recommended mixture is 1 volume of tri-*n*-butyl phosphate and 2 volumes of *iso*butyl methyl ketone; this should be made 2 per cent. with respect to hydrochloric acid as in the normal method.

No separation of platinum and palladium is possible however, since these two metals have similar R_F values in the tri-*n*-butyl phosphate - ketone mixture, but shorter columns may be used as a result, and separation from iridium[III] and rhodium is complete in about six extractions.

Iridium is then oxidised and extracted as before, but again, very few extractions suffice to complete the separation. The oxidising solvent is made up as described on p. 709 and the tri-*n*-butyl phosphate is added afterwards, as insufficient chlorine dioxide is formed if the tri-*n*-butyl phosphate is added first.

All the extracted metals can be recovered from the organic layer by stripping with 25 per cent. v/v nitric acid. Separating funnels are used for this operation and two extractions are sufficient. Any yellow colour remaining in the organic layer is not due to platinum metals and can be ignored.

The nitric acid solutions of the platinum metals are then evaporated to small volume and re-converted to chloride by boiling with an excess of hydrochloric acid. Separation of platinum and palladium must then be carried out with use of dimethylglyoxime in the conventional manner.

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The Effect of Oxygen on the Determination of 17:21-Dihydroxy-20-Oxosteroids with Tetrazolium Salts

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It has been shown that atmospheric oxygen affects the intensity and stability of the colour produced when 17:21-dihydroxy-20-oxosteroids are allowed to react with triphenyltetrazolium chloride. The influence of other factors has been re-examined, and a method for colour development is recommended. Tetrazolium blue has also been used as a reagent and is critically compared with triphenyltetrazolium chloride.

A METHOD for determining 17:21-dihydroxy-20-oxosteroids, based on the reduction of a tetrazolium salt to give a coloured formazan, was suggested by Mader and Buck.[1] Factors affecting the method have been studied by various workers,[2,3] and it is now the basis of some official assays.[4,5] During the application of the method to a standard hydrocortisone, it was noted that the variation obtained was greater than might have been expected and that this variation was apparently affected by the size of the vessel in which the colour was developed. This suggested that the presence of air, a factor not previously considered, may affect the assay, and this was subsequently confirmed. A re-examination of the conditions for colour development therefore seemed desirable.

The experiments described in this section were carried out on a standard solution of hydrocortisone in aldehyde-free ethanol. This solution contained 229 μg of hydrocortisone per 10 ml and was freshly prepared before use. The apparatus and reagents used are described under "Method," p. 718.

EFFECT OF AIR—

In order to examine the effect of air, other factors known to influence the reaction were rigidly controlled. The basic method used is described below.

To a solution of the steroid in 10 ml of aldehyde-free dehydrated ethanol in a 25-ml calibrated flask made of non-actinic glass add 2·0 ml of triphenyltetrazolium chloride reagent solution and then 2 ml of tetramethylammonium hydroxide reagent solution. Mix, and place the flask in a water bath at 30° C for a suitable time. Cool the flask and its contents rapidly to 20° C, and dilute to 25 ml with aldehyde-free ethanol. Gently shake, and immediately measure the optical density at 485 mμ in a 1-cm cell. Use 10 ml of aldehyde-free dehydrated ethanol, similarly treated, as blank solution.

Colours were developed from portions of the standard hydrocortisone solution for different periods in flasks containing nitrogen, air or oxygen. Fig. 1 shows the marked effect of the presence of oxygen in decreasing the maximum optical density and on the stability of the colour. This effect was shown to occur with solutions of cortisone, prednisone, prednisolone, hydrocortisone, the corresponding acetates, methylhydrocortisone and fludrocortisone.

The results for four typical examples are shown in Table I; the samples used were commercial production materials and were not pre-treated in any way. It can be seen that, for the free alcohols, maximum colour development in an atmosphere of nitrogen was attained within 45 minutes and that the solution was then stable for at least a further 2 hours. For the acetates, the colour took longer to develop, and for these a time of 60 minutes was used.

TABLE I

EFFECT OF AIR ON THE REACTION

The concentrations of the solutions of hydrocortisone, hydrocortisone acetate, prednisolone and fludrocortisone used were 229, 265, 228 and 242 μg per 10 ml, respectively

	Optical density for hydrocortisone—		Optical density for hydrocortisone acetate—		Optical density for prednisolone—		Optical density for fludrocortisone—	
Time for colour development	in air	in atmosphere of nitrogen	in air	in atmosphere of nitrogen	in air	in atmosphere of nitrogen	in air	in atmosphere of nitrogen
15 minutes	0·354	0·359	0·342	0·362	0·344	0·353	0·350	0·365
25 minutes	0·367	0·388	0·351	0·377	0·340	0·362	0·354	0·390
35 minutes	0·362	0·392	0·354	0·384	0·336	0·364	0·348	0·396
45 minutes	0·360	0·393	0·348	0·388	0·331	0·365	0·344	0·399
55 minutes	—	—	—	0·397	—	—	—	—
65 minutes	0·353	0·394	0·344	0·397	0·321	0·365	0·335	0·401
45 minutes and then set aside for 2 hours at room temperature	0·327	0·393	0·316	0·397	0·293	0·362	0·298	0·389

Difficulties were encountered with hydrocortisone sodium succinate and triamcinolone, colour development being incomplete after 4 hours (see Fig. 2). The anomalous results for triamcinolone are in agreement with the findings of Smith and Halwer.[6]

In the investigation of other factors, the basic method described above was used and colour was developed in an atmosphere of nitrogen for 45 minutes.

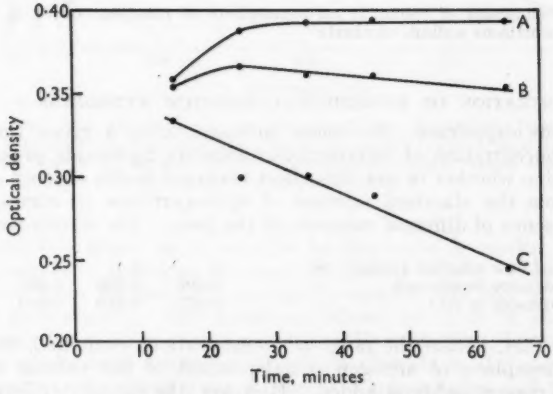

Fig. 1. Relationship between optical density of the hydrocortisone - triphenyltetrazolium colour and time under different conditions: curve A, in nitrogen; curve B, in air; curve C, in oxygen

EFFECT OF WATER—

It has been shown that the presence of water decreases the intensity of colour produced.[2] Since small amounts of water are unavoidably introduced in the tetramethylammonium hydroxide reagent solution and in the preparation of the aldehyde-free ethanol, experiments were carried out with the standard hydrocortisone solution to determine what effect, if any, this water had on the reaction. The water content of the aldehyde-free ethanol was determined by the Karl Fischer method, and, after allowance had been made for the water added in the tetramethylammonium hydroxide solution, water was added to bring the concentrations in the final solutions to the desired values. The results, from which it can be seen that the small amount of water introduced during the assay does not affect the colour intensity, were—

Water present, %	1·4	2·8	5·6	11·2
Optical density	0·392	0·394	0·392	0·368

EFFECT OF LIGHT—

During colour development, the solution must be protected from light,[2,3,4,5] otherwise there are rapid increases in the colours of the test and reagent blank solutions. We found that the reaction was also sensitive to light when carried out in an atmosphere of nitrogen.

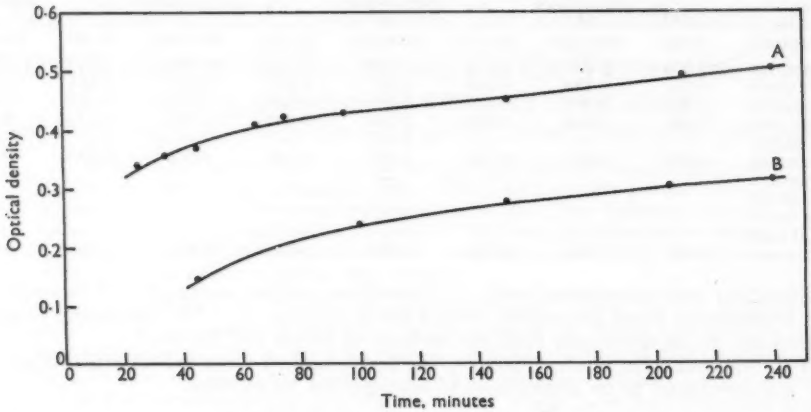

Fig. 2. Development of colour in an atmosphere of nitrogen: curve A, triamcinolone; curve B, hydrocortisone sodium succinate

EFFECT OF CONCENTRATION OF TETRAMETHYLAMMONIUM HYDROXIDE—

In our previous experience, the colour intensity after a given time decreased considerably as the concentration of tetramethylammonium hydroxide present was increased. In order to determine whether or not this effect occurred in the absence of oxygen, colours were developed from the standard solution of hydrocortisone in atmospheres of air and nitrogen in the presence of different amounts of the base. The results were—

Volume of base solution present, ml ..	1	2	3	4
Optical density in nitrogen	0·396	0·396	0·395	0·394
Optical density in air	0·377	0·360	0·354	0·333

These results show that, within the range of concentrations examined, the colour intensity produced in an atmosphere of nitrogen is independent of the volume of tetramethylammonium hydroxide reagent solution added. However, the colour developed under nitrogen in the solution containing 4 ml of base solution tended to fade while the optical density was being measured. It appears that the effect of air is accentuated by increasing the amount of base present.

CONSIDERATION OF ALTERNATIVE REAGENT—

Mader and Buck[1] examined 2:3:5-triphenyltetrazolium chloride and 3:3'-dianisole-bis-4:4'-(3:5-diphenyl)tetrazolium chloride (tetrazolium blue), and the latter reagent has been used to some extent, particularly by American workers.[6,7] The main advantage reported for it is greater sensitivity (about twice that of triphenyltetrazolium chloride), although this is only useful when trace amounts of steroid are determined. Colours were developed from hydrocortisone solutions in atmospheres of air and nitrogen by the basic method, but a 0·5 per cent. w/v solution of tetrazolium blue in aldehyde-free dehydrated ethanol was used instead of triphenyltetrazolium chloride. The optical densities of the solutions were read at 10-minute intervals against a blank solution developed for the same time. For 10-ml portions of a solution containing 17·2 mg of hydrocortisone per litre, the results were—

Time, minutes	5	15	25	35	65
Optical density in nitrogen ..	—	0·450	0·460	0·452	0·460
Optical density in air	0·371	0·445	0·449	0·444	0·464

It can be seen that maximum colour developed more rapidly than when triphenyltetrazolium chloride solution was used and that the effect of oxygen was less marked. Despite these facts, however, the reproducibility of the optical density in an atmosphere of nitrogen was poor compared with that obtained when triphenyltetrazolium chloride solution was used (see Table I). It was observed that an intensely coloured reagent blank solution was obtained for tetrazolium blue, whereas that for triphenyltetrazolium chloride was practically colourless.

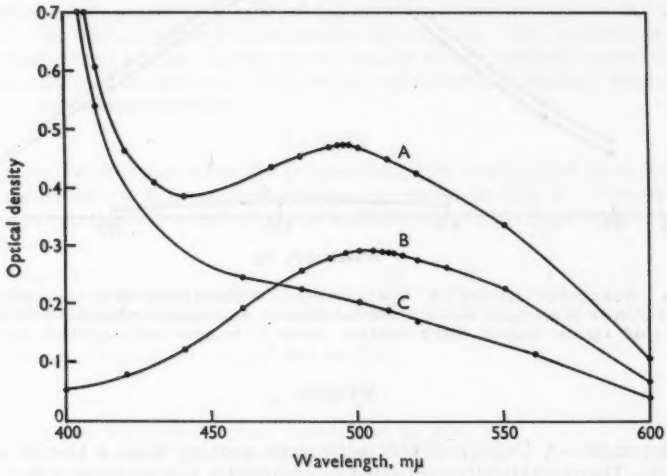

Fig. 3. Absorption spectra of hydrocortisone - tetrazolium blue colour in an atmosphere of nitrogen: curve A, steroid solution read against ethanol; curve B, steroid solution read against reagent blank solution; curve C, reagent blank solution read against ethanol

It was thought that this might be responsible for the variable results, and the behaviour of the blank solutions was therefore examined in greater detail. Colours were developed for different periods from solutions containing no steroid, and the optical densities were measured against pure ethanol. The results are shown in Table II and indicate that the colour produced with tetrazolium blue increases rapidly with time; for comparison, a corresponding series of results with triphenyltetrazolium chloride is also shown.

Absorption spectra were plotted for a reagent blank solution and a standard solution of hydrocortisone; tetrazolium blue reagent solution was used, and colour was developed in an atmosphere of nitrogen. These curves were compared with those obtained when triphenyltetrazolium chloride was used as reagent (see Figs. 3 and 4). Triphenyltetrazolium

TABLE II

RATE OF COLOUR DEVELOPMENT IN REAGENT BLANK SOLUTIONS

Time, minutes	Optical density of colour produced with tetrazolium blue— in air	Optical density of colour produced with tetrazolium blue— in atmosphere of nitrogen	Optical density of colour produced with triphenyltetrazolium chloride in atmosphere of nitrogen
15	0·132	0·124	0·020
25	0·145	0·150	0·022
35	0·167	0·170	0·024
45	0·184	0·188	0·026
65	0·215	0·221	0·028

chloride is obviously a better reagent from this point of view, and, in our opinion, it is a more satisfactory reagent for the routine assay of ketosteroids than is tetrazolium blue. The greater sensitivity and the freedom from interference by oxygen of the latter reagent are more than outweighed by the high and continuously varying blank readings.

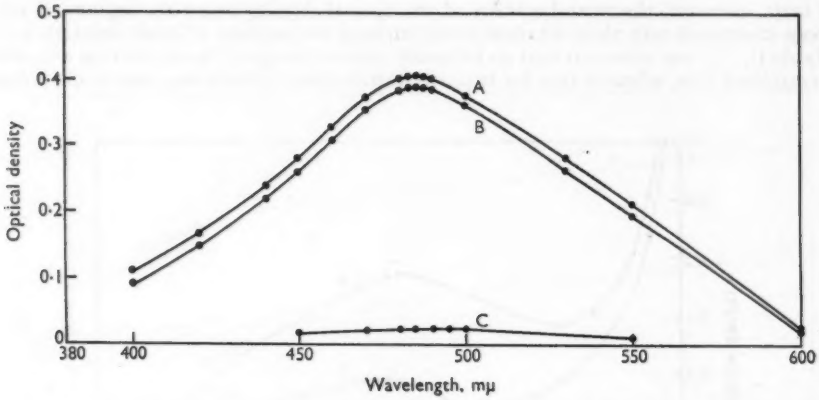

Fig. 4. Absorption spectra of hydrocortisone - triphenyltetrazolium chloride colour in an atmosphere of nitrogen: curve A, steroid solution read against ethanol; curve B, steroid solution read against reagent blank solution; curve C, reagent blank solution read against ethanol

METHOD

APPARATUS—

Spectrophotometer—A Unicam SP600 instrument working from a 12-volt battery.

Water bath—Thermostatically controlled to maintain a temperature of 30° ± 0·5° C and fitted with a cover to exclude light.

REAGENTS—

Ethanol, aldehyde-free, dehydrated.

Triphenyltetrazolium chloride reagent solution—Dissolve 0·1 g of 2:3:5-triphenyltetrazolium chloride in 20 ml of aldehyde-free dehydrated ethanol. This solution should be freshly prepared and kept protected from light.

Tetramethylammonium hydroxide reagent solution—Dilute 4 ml of a 25 per cent. w/v aqueous solution of tetramethylammonium hydroxide to 100 ml with aldehyde-free dehydrated ethanol. Filter the solution before use.

PROCEDURE—

By pipette, place 10 ml of aldehyde-free dehydrated ethanol containing 120 to 450 μg of steroid in a 25-ml calibrated flask made of non-actinic glass. Add 2·0 ml of triphenyltetrazolium chloride reagent solution, and displace the air from the flask with a stream of

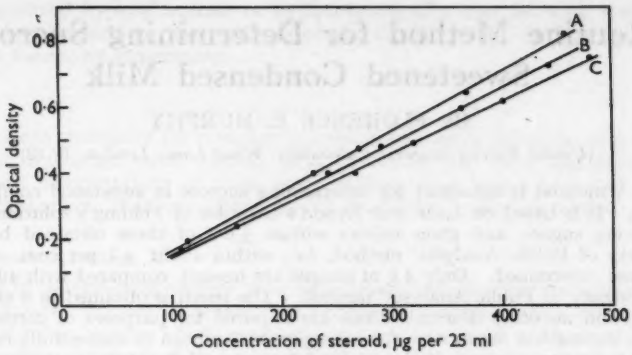

Fig. 5. Typical calibration graphs: curve A, hydrocortisone; curve B, fludrocortisone; curve C, prednisone acetate

oxygen-free nitrogen. (At this stage, the flask should be stoppered until the base solution is added.) Add 2·0 ml of tetramethylammonium hydroxide reagent solution, and again displace air from the flask. Gently swirl to mix the contents of the flask, and place in a water bath at 30° C for 1 hour. Cool the flask and its contents rapidly, and dilute to the mark with aldehyde-free dehydrated ethanol (all solutions were brought to 20° C to obviate errors caused by the high coefficient of expansion of ethanol). Mix by shaking gently, and immediately measure the optical density at 485 mµ in closed optically matched 1-cm cells against a blank solution consisting of 10 ml of aldehyde-free dehydrated ethanol treated in the same way as the sample solution.

Results

Calibration graphs obtained when the proposed method was applied to samples of hydrocortisone, fludrocortisone and prednisone acetate are shown in Fig. 5. The reproducibility of the method was found by means of a series of replicate determinations by different analysts; the results were—

Determination No.	1	2	3	4	5	6
Optical density found by analyst A ..	0·400	0·397	0·397	0·394	0·400	0·396
Optical density found by analyst B ..	0·399	0·397	0·400	—	—	—

Analyst A had no previous experience of the method.

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A Routine Method for Determining Sucrose in Sweetened Condensed Milk

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A method is described for determining sucrose in sweetened condensed milk. It is based on Lane and Eynon's titration of Fehling's solution with reducing sugars, and gives results within ±0·5 of those obtained by the Society of Public Analysts' method, *i.e.*, within about ±1 per cent. of the sucrose determined. Only 4 g of sample are needed, compared with 40 g for the Society of Public Analysts' method. The result is obtained in a shorter time, and no other determinations are required for purposes of correction. Since the method requires no special apparatus and can be successfully carried out by routine workers, it is suitable for use in a laboratory controlling the manufacture of condensed milk.

A METHOD of determining sucrose in sweetened condensed milk was required; it was to be suitable for use in a factory control laboratory. Such a method should be as rapid and simple as possible and should require no apparatus other than that usually found in this type of laboratory. The result should agree with that obtained by the method recommended by the Society of Public Analysts[1] (now The Society for Analytical Chemistry) to within ±0·5, *i.e.*, about ±1 per cent. of the sucrose found. Consideration of the problem led to the conclusion that a copper-reduction method might be applicable, the sucrose content being calculated from the increased reducing power obtained by inversion. As condensed milk contains about 45 per cent. of sucrose and reduction methods require dilute solutions of sugar, small errors due to technique or to interfering substances must be avoided. Further, the chosen method of inversion must hydrolyse sucrose and leave lactose unchanged.

VALIDITY OF COPPER-REDUCTION METHOD—

Preliminary work showed that the copper-reducing powers of lactose and inverted sucrose are additive under the conditions of Lane and Eynon's titration.[2] Previous work on the determination of lactose in milk and milk products had confirmed the statement of these workers that stricter adherence to the same conditions is necessary when titrating with solutions of lactose than with solutions of most other reducing sugars. The strict technique found necessary and adopted in this laboratory was used in this work.

APPLICATION TO CONDENSED MILK—

Titrations carried out with condensed milk sera obtained by clarification with lead acetate and potassium oxalate and with the same sera after inversion of the sucrose by Clerget's method gave results in close agreement with those of the S.P.A. method. Although this provides a useful alternative to the polarimetric method, it is too lengthy, and the conditions for the Clerget inversion are difficult to maintain in a control laboratory.

DEVELOPMENT OF ROUTINE METHOD

CLARIFICATION—

It was hoped to eliminate the lengthy preparation of a lead serum. Titrations were therefore carried out with unclarified dilutions of condensed milk and with the same dilutions inverted by Clerget's method and then further diluted to a suitable concentration. In 83 per cent. of the forty-one samples examined the results were within ±0·5 of those by the S.P.A. method; for the other samples the greatest difference between the results by the two methods was 1·1.

Calcium interferes with the Fehling's titration,[3] but the amount of ionisable calcium in a dilution of condensed milk was expected to be small[4,5] and to cause negligible interference. This appeared to be so for most of the samples examined, but interference from calcium was thought to be a possible cause of error when the difference between results by the reduction method and the S.P.A. method was greater than 0·5. Further experiments showed that, when the unclarified dilutions gave poor results, the agreement became satisfactory if the

calcium was removed by lead acetate or by formation of a complex with sodium hexametaphosphate (Calgon) added to the contents of the titration flask.[6] The optimum addition of Calgon was found by experiment.

INVERSION—

The conditions necessary for complete inversion of sucrose by citric acid without danger of break-down of lactose were studied, acid concentration, time and temperature being varied. Various combinations of these three factors were found to be effective, and the combination chosen was that considered most suitable for a routine method.

PRACTICAL DIFFICULTIES—

A method of determining sucrose in which unclarified dilutions were used and inversion was carried out with citric acid was tested in a control laboratory; the results were not always within $\pm 0{\cdot}5$ of those by the S.P.A. method. However, enough preliminary work had been done to show that the method was sound and capable of giving satisfactory results, and poor agreement was therefore attributed to difficulties experienced by routine workers. Failure to recognise the rather different end-points of the two titrations was probably the greatest difficulty.

Further work has shown that agreement is better if a simple method of clarification is adopted and if both titrations are completed with the inverted serum, which contains much more invert sugar than lactose. The first modification makes recognition of the end-point easier, and the second avoids the use of the two rather different end-points.

It has also been shown that there is a possibility of break-down of one or both sugars in these dilute solutions, particularly when the atmospheric temperature is high.

The preliminary experiments and the work subsequently carried out as a result of control-laboratory experience has led to the adoption of the method described below.

METHOD

PROCEDURE—

Weigh $4 \pm 0{\cdot}005$ g of well mixed sample into a small covered beaker, and wash it into a 250-ml calibrated flask with not more than 25 ml of hot distilled water. Add 0·5 ml of a 10 per cent. w/v solution of citric acid, mix by rotating the flask, cool, and dilute to the mark at 20° C with distilled water. Insert the stopper, invert the flask several times, and filter the contents into a clean dry conical flask, rejecting the first portion of filtrate. (This filtrate is solution A.) When enough filtrate has been collected and while it is still at 20° C, transfer a 50-ml portion, by pipette, to a 250-ml conical flask. Add 20 ml of distilled water, 30 ml of the citric acid solution and two or three glass beads, and close with a bulb stopper. Heat the flask over a covered flame until its contents begin to boil, lower the flame, and allow the liquid to boil gently for exactly 10 minutes. (Little diminution in volume should take place during the boiling period, as it is important that the concentration of acid should not increase appreciably.) Remove the flask from the source of heat, rinse the lower end of the bulb stopper into the flask with distilled water, and cover the neck of the flask with an inverted small beaker. Cool the flask immediately and rapidly by allowing cold water to flow over the beaker and down the sides of the flask. When the flask has cooled to below 30° C, add a small piece of litmus paper to its contents, and slowly run in approximately 5 *N* sodium hydroxide, with thorough mixing, until the litmus paper just changes colour (about 9 ml of alkali will be needed). Transfer the solution to a 250-ml calibrated flask, and dilute to the mark at 20° C with distilled water. (This is solution B, in which the lactose is unchanged and the sucrose has been hydrolysed to invert sugar.)

Determine the reducing power of solution B by Lane and Eynon's method; use a burette with a well lubricated glass tap. Titrate the solution into a flask containing 10 ml of Fehling's solution, 5 ml of distilled water and a little powdered pumice. Add 23 ml of solution B as the first addition of the incremental titration if the sample is a full-cream milk and 21 ml if it is a separated milk. Use a measured amount (0·2 ml) of an aqueous 1 per cent. solution of methylene blue as indicator, and, when completing the titration, add 0·5-ml portions of solution B from the burette at 10-second intervals. When completing the standard titration add 0·1-ml portions of sugar solution from the burette at 10-second intervals;

these portions should total not less than 0·5 ml and not more than 0·9 ml. The total boiling period should not exceed 3½ minutes (in either titration).

Determine the reducing power of solution A. In the titration flask containing 10 ml of Fehling's solution place, by pipette, 25 ml of solution A if the sample is a full-cream milk and 20 ml if it is a separated milk. Add 5 ml of a freshly prepared 10 per cent. w/v solution of Calgon, and carry out an incremental titration by adding solution B from the burette. Carry out a standard titration in a similar manner.

The titrations should be carried out as soon as the sera have been prepared. Only one sample should be analysed at a time when the atmospheric temperature exceeds 70° F.

Calculation of results

In a particular determination, the volumes of sugar solutions equivalent to 10 ml of Fehling's solution were 29·0 ml of solution B and 25·0 ml of solution A *plus* 8·5 ml of solution B. An 8·5-ml portion of solution B therefore has the same reducing power as 10·35 ml of solution A, and a titration completed with solution A would require a total of 35·35 ml. The factor for the Fehling's solution was 0·996, so that the amounts of solutions A and B equivalent to 10 ml of Fehling's solution (F = 1·000) were 35·5 and 29·1 ml, respectively.

From Lane and Eynon's Tables for invert sugar, 10 ml of Fehling's solution would be reduced by (*a*) 35·5 ml of a solution containing 145·9 mg of invert sugar per 100 ml and (*b*) 29·1 ml of a solution containing 177·0 mg of invert sugar per 100 ml. The reducing powers of solutions A and B were therefore equivalent to concentrations of 145·9 and 177·0 mg of invert sugar per 100 ml, respectively.

Solution B was produced from solution A by inversion and dilution (1 + 4), so that the increase in reducing power produced by inversion was equivalent to a concentration of [(177·0 × 5) — 145·9] mg of invert sugar per 100 ml, *i.e.*, 739·1 mg per 100 ml. This concentration is equivalent to $\frac{739{\cdot}1 \times 5}{2}$ mg of invert sugar per 250 ml of the solution containing 4 g of sample. The percentage of invert sugar produced from the sample was therefore given by $\frac{5 \times 739{\cdot}1 \times 100}{2 \times 4 \times 1000}$ and this is equivalent to $\frac{5 \times 739{\cdot}1 \times 100 \times 0{\cdot}95}{2 \times 4 \times 1000}$ per cent. of sucrose, *i.e.*, the sucrose content of the sample was 43·9 per cent.

In this calculation, no correction has been made for the presence of sucrose[7] in solution A. Such a correction would amount to about 0·1 per cent. of sucrose, and, since this is well within the limits of agreement required, it has been disregarded.

As an aid to rapid working in routine determinations, sucrose contents have been calculated as described above for pairs of whole-number titres over the range normally encountered. The results are shown in Table I, and sucrose contents for other than whole-number titres can be obtained by interpolation.

Table I

Sucrose contents corresponding to various titres

Volume of solution A equivalent to 10 ml of Fehling's solution, ml	Sucrose content of sample when 10 ml of Fehling's solution are equivalent to—							
	26 ml of solution B, %	27 ml of solution B, %	28 ml of solution B, %	29 ml of solution B, %	30 ml of solution B, %	31 ml of solution B, %	32 ml of solution B, %	33 ml of solution B, %
26	46·9	44·8	42·8	41·0	39·2	—	—	—
27	47·3	45·2	43·2	41·4	39·7	—	—	—
28	47·7	45·6	43·6	41·8	40·1	38·5	—	—
29	48·1	46·0	44·0	42·2	40·4	38·8	—	—
30	48·4	46·3	44·4	42·5	40·8	39·1	—	—
31	—	—	44·7	42·9	41·1	39·4	—	—
32	—	—	45·0	43·1	41·4	39·7	38·3	—
33	—	—	45·2	43·4	41·7	40·0	38·6	—
34	—	—	45·5	43·7	41·9	40·3	38·9	—
35	—	—	45·8	44·0	42·2	40·6	39·1	—
36	—	—	46·0	44·2	42·4	40·8	39·3	37·9

Twenty-five determinations on nineteen samples were carried out by the proposed method. The difference between each pair of results for the six duplicate determinations was not more than 0·3 per cent. of sucrose. The figures below show how the results compared with those found by the S.P.A. method.

Difference from result by S.P.A. method, %	±0·1	±0·2	±0·3	±0·4	±0·5	±0·6
Number of results with this difference ..	9	6	4	3	2	1

I thank Mr. S. Schimelmitz for supplying the sucrose figures determined by the S.P.A. method and for carrying out the reduction method on some of the samples.

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Polynuclear Hydrocarbons in Tobacco and Tobacco Smoke

Part II.* The Origin of 3:4-Benzopyrene Found in Tobacco and Tobacco Smoke

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It is shown that the 3:4-benzopyrene present on unsmoked cigarette tobacco derives from contamination occurring during processing and that the presence of the hydrocarbon in cigarette smoke is due mainly to synthesis in the burning cigarette.

In Part I of this series,[1] methods were described for determining 3:4-benzopyrene in leaf, cigarette tobacco and tobacco smoke. The results reported showed that unsmoked cigarette tobacco contains 3:4-benzopyrene in amounts approximately equal to the amounts of the hydrocarbon found in the main-stream smoke from an equal weight of cigarettes. Because polynuclear hydrocarbons are not natural constituents of plant tissues, the presence of 3:4-benzopyrene in unsmoked tobacco must be caused by contamination. It was therefore of interest to discover at what stage between planting tobacco seedlings and the end of the manufacturing process this contamination arises.

Any 3:4-benzopyrene present on tobacco will not be there as the pure hydrocarbon, but as part of the complex mixture of substances that together comprise the particles of soot and dust adhering to the surface of the leaf; chimney soot, for example, contains 0·03 per cent. w/w of 3:4-benzopyrene.[2]

Most of the flue-cured tobacco used for manufacture in the United Kingdom is grown in areas where contamination due to atmospheric pollution is unlikely to be large. In the flue-curing process, however, the leaf may be exposed to gases escaping from faulty flues, and these gases, being the products of the incomplete combustion of wood, coal or oil, are a possible source of contamination with 3:4-benzopyrene. In the subsequent re-drying process, large volumes of air are blown through the cured leaf, and the leaf is thereby exposed to further possible contamination by atmospheric soot and dust.

Experiments were therefore designed to discover the extent to which freshly harvested tobacco leaf becomes contaminated with 3:4-benzopyrene during the flue-curing and re-drying processes. At the same time, we studied the possible effects of two innovations in curing technology, namely, the use of steam-curing equipment in Rhodesia and of open-flame

* For details of Part I of this series, see reference list, p. 727.

oil burners in the U.S.A. It was thought that steam-curing, in which the flues are heated by steam instead of hot combustion gases, might lead to less contamination than is normal. Curing by the use of open-flame oil burners, in which the leaf is exposed directly to the products of combustion of kerosene, was thought likely to result in greatly increased contamination with 3:4-benzopyrene, particularly when burners were badly adjusted.

TABLE I

3:4-BENZOPYRENE CONTENTS OF TOBACCO SAMPLES AT DIFFERENT STAGES OF PROCESSING

Type of tobacco	Series No.		Description of sample	Maximum 3:4-benzopyrene content per 500 g of sample, μg
Rhodesian	1	(a)	Green (uncured) tobacco	Not found
		(b)	Normal flue-cured tobacco, not re-dried	Not found, 0·5
		(c)	Normal flue-cured tobacco, re-dried	4·0, 2·5
	2	(a)	Green (uncured) tobacco	2·5, 3·5
		(b)	Normal flue-cured tobacco, not re-dried	1·0, Not found
		(c)	Steam-cured tobacco, not re-dried	2·0, 0·5
American	3	(a)	Green (uncured) tobacco	Not found
		(b)	Normal flue-cured tobacco, not re-dried	0·7, Not found
		(c)	Normal flue-cured tobacco, re-dried	1·5, 3·0
	4	(a)	Green (uncured) tobacco	Not found
		(b)	Normal flue-cured tobacco, not re-dried	1·5, 0·5
		(c)	Tobacco cured with open-flame oil burners, not re-dried	1·25, 1·0
American; cured with open-flame burners, not re-dried	5	(a)	Heavily contaminated with burner-oil soot	20, 15
		(b)	Lightly contaminated	6, 6
		(c)	No visible contamination	Not found
Manufactured cigarette tobacco ..	6	(a)		0·5, 1·0
		(b)		6·0
		(c)		2·5, 1·25

Several series of tobacco samples carefully selected at different stages of the curing and re-drying processes were obtained from Rhodesia and the U.S.A. Immediately after selection, each sample was sealed in a polythene bag to prevent further contamination during the journey to the United Kingdom. Table I shows the results found when these samples were analysed for 3:4-benzopyrene by the method described previously.[1] The accuracy of the determinations of 3:4-benzopyrene content was within about ±20 per cent.

Within the limits of experimental error, the results from the two comparable series, Nos. 1 and 3, are in broad agreement. The indications are that in both Rhodesia and the U.S.A. freshly harvested tobacco grown in areas remote from large towns contains no detectable amount of 3:4-benzopyrene. When cured by conventional means, and more especially when re-dried, contamination with 3:4-benzopyrene increases stepwise to approximately the level found on leaf imported into the United Kingdom.

The uncured leaf in series No. 2 was exceptional in being appreciably contaminated with 3:4-benzopyrene. However, within the limits of experimental error, there is no difference between the levels of contamination found on steam-cured leaf and on leaf cured by conventional means.

Series No. 4 is a comparison of the levels of contamination found on commercially acceptable leaf cured by conventional means and by open-flame oil burners. There is no significant difference between the amounts of 3:4-benzopyrene found on leaf cured by the two methods. Series No. 5 shows, however, that large amounts of 3:4-benzopyrene are found in leaf cured by means of badly adjusted oil burners.

Series No. 6 shows the levels of 3:4-benzopyrene found on typical samples of manufactured cigarette tobacco.

It can be seen that, between the harvesting of the tobacco and its final appearance as manufactured cigarettes, there is a stepwise increase in contamination by 3:4-benzopyrene. This is shown below, the figures in parenthesis being the 3:4-benzopyrene contents per 500 g of material.

Green leaf → Flue-cured leaf → Re-dried leaf → Manufactured product
(Nil) (1 μg) (3 μg) (3·5 μg)

It was of further interest to discover whether the 3:4-benzopyrene found in cigarette smoke arises by direct transfer to the smoke of the hydrocarbon originally present on the cigarette tobacco or by synthesis *de novo* in the complex processes that occur immediately behind the burning zone of a cigarette.

If cured and re-dried tobacco leaf entirely free from contamination by 3:4-benzopyrene could be obtained, then the problem could be resolved simply by measuring the 3:4-benzopyrene content of the smoke from cigarettes made from such leaf. This approach is, however, impracticable, since one can never be certain of the exact amount of 3:4-benzopyrene on any particular sample of leaf. This is because there is no means of ascertaining the analytical recovery of 3:4-benzopyrene adsorbed on particles of soot and dust. The apparent 3:4-benzopyrene content of leaf represents an unknown fraction of the true amount present. This is not so for cigarette-smoke condensate, since the analytical recovery of 3:4-benzopyrene can be determined directly by addition experiments.

We therefore measured the recovery in cigarette smoke of pure 3:4-benzopyrene added to manufactured tobacco in amounts so large compared with the normal levels of contamination that the effects of the latter could be disregarded.

Cigarettes were made from tobacco to which 3:4-benzopyrene had been added at the level of 100 μg per 500 g of leaf. These cigarettes were smoked under standard conditions,[1] and the main-stream smoke, side-stream smoke, stubs and ash were subsequently analysed for 3:4-benzopyrene. A parallel experiment was carried out with cigarettes made from similar tobacco to which no 3:4-benzopyrene had been added. The results are shown in Table II.

TABLE II

RECOVERY OF 3:4-BENZOPYRENE ADDED TO CIGARETTE TOBACCO

Material analysed	Maximum 3:4-benzopyrene content per 500 g of cigarettes containing—	
	added hydrocarbon, μg	no added hydrocarbon, μg
Main-stream smoke	15·0	7·0
Side-stream smoke	25·0	15·0
Stubs	17·0	5·0
Ash	Not found	Not found

If it is assumed that no 3:4-benzopyrene is synthesised in the smoking process, then, for leaf containing 100 μg of added hydrocarbon per 500 g, the recovery of added hydrocarbon in the main-stream smoke is 15 per cent. The addition of the pure hydrocarbon to simulate contamination provides the optimum conditions for its recovery in smoke. The recovery of 3:4-benzopyrene from normal leaf will be less than 15 per cent., as the hydrocarbon is adsorbed on particles of soot and dust and is therefore more likely than the added pure hydrocarbon to be decomposed rather than distilled. For the same reason, the analytical method underdetermines to an unknown extent the amount of 3:4-benzopyrene present on leaf naturally contaminated with the hydrocarbon. The recovery of the pure hydrocarbon from leaf by extraction with acetone and subsequent chromatography[1] is 75 to 80 per cent.; the recovery of 3:4-benzopyrene adsorbed on soot and dust and present on the leaf as natural contamination may be of the order of 50 per cent., but is unlikely to be much lower.

From results reported earlier[1] and those found during this work, groups can be selected when figures are available for unsmoked material and the smoke derived from it (see Table III).

For comparison, it is reasonable to double the figures for 3:4-benzopyrene in unsmoked tobacco to allow for the low recovery of the hydrocarbon. In Table IV, these corrected figures are compared with the probable amounts of 3:4-benzopyrene present in all the products of combustion, namely, main-stream smoke, side-stream smoke, stubs and ash. These figures for total 3:4-benzopyrene are derived by multiplying the main-stream values shown in Table III by 4 (see Table II).

It is therefore obvious that, when tobacco, tobacco stems and cigarette paper are burned, appreciable amounts of 3:4-benzopyrene are synthesised. This means that the apparent recovery (15 per cent.) of added 3:4-benzopyrene, calculated from Table II, is too high, since it was assumed that none of the hydrocarbon was synthesised during burning. Therefore, although there is no unequivocal proof, it is reasonable to presume that not more than, say,

TABLE III

3:4-BENZOPYRENE CONTENTS OF UNSMOKED MATERIAL AND SMOKE DERIVED THEREFROM

Material	Average 3:4-benzopyrene content of—	
	500 g of unsmoked material, μg	main-stream smoke derived from 500 g of material, μg
Mixed cigarettes	3·0	3·0
Single commercial blend	3·4	5·2
Air-cured tobacco	0·3	0·8
Flue-cured tobacco	2·5	3·3
Flue-cured tobacco, not re-dried	0·3	4·2
Tobacco stems	0·2	4·5
Paper cigarettes*	—	10·0

* Cigarettes made from finely cut cigarette paper and smoked under the standard conditions used.

10 per cent. of the 3:4-benzopyrene present as contamination is transferred to the smoke. The appearance of 3:4-benzopyrene in smoke can thus be represented by the "equation" below, which shows the contributions due to synthesis, to contamination and to combustion of the paper; the figures refer to amounts of 3:4-benzopyrene per 500 g of cigarettes.

Combustion of paper (0·4 μg)	+	Contamination (0·4 μg)	+	Synthesis during burning (4·2 μg)	→	Main-stream smoke (5 μg)

There also seems to be no correlation between the amounts of 3:4-benzopyrene appearing in main-stream smoke and the amounts present on the unsmoked material. It follows then that, if the general level of contamination of manufactured cigarette tobacco by 3:4-benzopyrene were reduced to, say, one-tenth of its present level, the effect on the amount of the hydrocarbon appearing in main-stream smoke would be negligible. Reduction of the amounts of 3:4-benzopyrene appearing in main-stream smoke therefore depends upon the discovery of means whereby the synthesis of the hydrocarbon in the burning cigarette can be effectively reduced or whereby the hydrocarbon, once formed, can be destroyed.

METHOD

3:4-Benzopyrene was determined in main-stream smoke and in tobacco by the methods already described.[1] Side-stream smoke and ash were collected by the apparatus shown in Fig. 1.

The precipitator tube, A, of the automatic smoking machine was fitted with a cigarette holder, B, shaped to fit a wide glass tube, C, fitted with a B34 socket and a side-arm, D. A gentle stream of air, just sufficient to prevent smoke escaping from the open end of C, was

TABLE IV

COMPARISON BETWEEN 3:4-BENZOPYRENE CONTENTS OF UNSMOKED MATERIAL AND PRODUCTS OF COMBUSTION

Material	Average 3:4-benzopyrene content of—	
	500 g of unsmoked material (corrected), μg	all products of combustion from 500 g of material (calculated total), μg
Mixed cigarettes	6·0	12·0
Single commercial blend	6·8	20·8
Air-cured tobacco	0·6	3·2
Flue-cured tobacco	5·0	13·2
Flue-cured tobacco, not re-dried	0·6	16·8
Tobacco stems	0·4	18·0

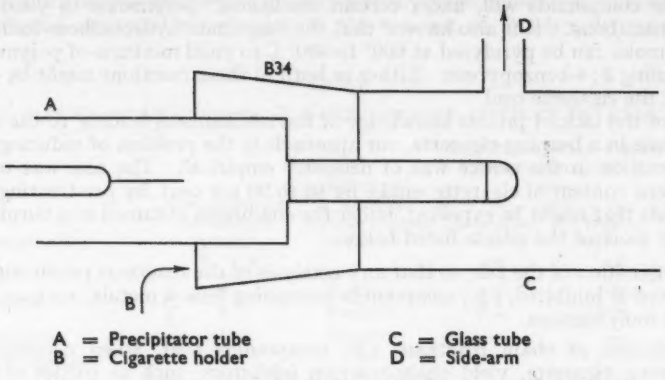

Fig. 1. Arrangement for collecting side-stream smoke and ash

drawn through side-arm D, and the smoke thereby removed from C was collected by means of two traps in series, packed with glass-wool and cooled to −70° C. The smoke condensate was finally collected by means of acetone and analysed for 3:4-benzopyrene as before.[1]

Ash that collected in tube C was extracted with acetone, and the extract was subsequently analysed for 3:4-benzopyrene.

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NOTE—Reference 1 is to Part I of this series.

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Polynuclear Hydrocarbons in Tobacco and Tobacco Smoke

Part III.* The Inhibition of the Formation of 3:4-Benzopyrene in Cigarette Smoke

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The amounts of 3:4-benzopyrene appearing in cigarette smoke can be reduced by up to 80 per cent. by pre-treating the tobacco with a suitable additive. Pre-treatment of the paper on a cigarette with ammonium sulphamate does not reduce the amount of 3:4-benzopyrene in the main-stream smoke.

IT has been shown in Part II of this series[1] that there are compelling reasons for presuming that most of the 3:4-benzopyrene found in cigarette smoke is formed in the pyrolytic reactions taking place in the burning cigarette. Although the precise nature of these reactions is a matter for conjecture, it is most probable that chain reactions involving free radicles and reactions involving the dehydrogenation and cyclisation of long-chain paraffinic and olefinic hydrocarbons are mainly responsible for the production of 3:4-benzopyrene. It is known[2,3] that cigarette smoke contains small amounts of methane, acetylene, ethylene and isoprene

* For details of Parts I and II of this series, see reference list, p. 730.

and that these compounds will, under certain conditions,[4] polymerise to yield polynuclear aromatic hydrocarbons. It is also known[5] that the long-chain hydrocarbons found in tobacco and tobacco smoke can be pyrolysed at 600° to 900° C to yield mixtures of polynuclear hydrocarbons, including 3:4-benzopyrene. Either or both of these reactions might be catalysed by the hot ash in the cigarette coal.

Because of the lack of precise knowledge of the mechanisms leading to the formation of 3:4-benzopyrene in a burning cigarette, our approach to the problem of reducing the amount of this hydrocarbon in the smoke was of necessity empirical. The aim was to reduce the 3:4-benzopyrene content of cigarette smoke by 80 to 90 per cent. by pre-treating the tobacco with compounds that might be expected, under the conditions obtained in a burning cigarette, to have one or more of the effects listed below.

(*i*) Modification of the ash, so that any catalysis of the reactions producing 3:4-benzopyrene is inhibited, *e.g.*, compounds containing heavy metals, such as iron, cobalt and molybdenum.

(*ii*) Inhibition of chain reactions, *e.g.*, compounds that, when decomposed in the burning cigarette, yield chain-reaction inhibitors, such as oxides of nitrogen or radicles containing nitrogen, for example, =NH or $—NH_2$.

(*iii*) Production in the smoke of an excess of oxygen or oxidising agents that could possibly destroy any 3:4-benzopyrene formed, *e.g.*, compounds such as potassium nitrate, bromate or persulphate or organic peroxides.

(*iv*) Lowering of the coal temperature of burning cigarettes to a level at which the production of 3:4-benzopyrene is minimal; this might be achieved by using a high moisture content or by pre-treating the tobacco with compounds, such as glycerol or ethylene glycol, that form comparatively large amounts of water when burnt.

The method of testing was partly empirical. Fluorescence spectroscopy, as described in Part I of this series,[6] was used; when preliminary examination of the plates showed no evidence of any appreciable reduction of 3:4-benzopyrene compared with controls, the analytical procedure was not completed. The compounds tested are listed in Table I.

The compounds tested can be divided into several groups according to the manner in which they might be expected to effect a reduction in the 3:4-benzopyrene in cigarette smoke. Four such groups are—

(*i*) Salts containing heavy metals.

(*ii*) Organic compounds that could yield chain inhibitors when decomposed.

(*iii*) Oxidising agents or substances yielding oxidising agents when decomposed.

(*iv*) Compounds yielding relatively large amounts of water on combustion.

Some of the compounds tested could belong to more than one of these groups, so that any reduction in the 3:4-benzopyrene content of smoke cannot be correlated with a particular property of any such compound. For example, all the nitrates tested, with the exception of silver nitrate, would decompose in the cigarette coal to yield the metal oxide, oxygen and oxides of nitrogen. All of these except potassium nitrate contain a heavy metal, so that any effect could be due to the heavy-metal oxide, oxidation of the hydrocarbon after formation or prevention of its formation by the inhibiting action of oxides of nitrogen.

Of the oxidising agents tested, only potassium nitrate had any effect. Of the other nitrates tested, only the copper salt was effective, and the best results were obtained when this compound was present at levels of 2·5 and 5 per cent.; the effect at the 1 per cent. level was somewhat less. The fact that copper sulphate and other metal nitrates had no effect suggests that the results obtained with copper nitrate are due to some unique property of that compound. For example, the molecule of anhydrous copper nitrate can exist undissociated in the vapour phase.[7]

Halides are known to inhibit certain chain reactions,[8] but the potassium halides tested had no effect.

Aniline, urea and phenol were tested, as on combustion these compounds were likely to yield radicles that might interfere with the reactions involved in the formation of 3:4-benzopyrene; none of them had any effect.

Glycerol and ethylene glycol give rise to relatively large amounts of water on combustion, and both effected appreciable reduction in the amount of 3:4-benzopyrene appearing in smoke.

TABLE I

EFFECTS OF VARIOUS COMPOUNDS ON 3:4-BENZOPYRENE CONTENT OF TOBACCO SMOKE

Compound	Concentration present on tobacco, % w/w	Effect on 3:4-benzopyrene content of smoke*	3:4-Benzopyrene content of smoke from 500 g of— untreated tobacco, μg	3:4-Benzopyrene content of smoke from 500 g of— treated tobacco, μg	Reduction in 3:4-benzopyrene content of smoke, %
Potassium nitrate	2	Positive	4·5	1·95†	57
	4	Positive	5·5	1·8‡	67
Potassium bromate	4	Negative	—	—	—
Ammonium persulphate, Lead nitrate, Silver nitrate, Zinc nitrate	5	Negative	—	—	—
Copper nitrate	5	Positive	8·0§	1·3§	84
	5	Positive	6·2§	1·6§	74
	2·5	Positive	6·2§	1·3§	79
	1	Positive	6·2§	2·2§	64
Copper sulphate	5	Negative	—	—	—
Sodium nitrite	5	Positive	6·2§	1·7§	73
Ammonium ferrous sulphate, Ammonium ferric sulphate, Ammonium nickel sulphate, Ammonium cobalt sulphate, Ammonium chromic sulphate, Ammonium ceric sulphate, Ammonium molybdate, Potassium iodide, Potassium bromide, Potassium chloride, Sodium tetraborate	5	Negative	—	—	—
Ascorbic acid	0·5	Negative	—	—	—
Glycerol	3	Positive	5·5	2·1†	62
Ethylene glycol	3	Positive	5·5	2·4†	56
Aniline, Urea, Phenol	5	Negative	—	—	—

* "Negative" indicates that there was no evidence of reduction in the 3:4-benzopyrene content in the preliminary stages of the determination.
† Average of four determinations.
‡ Average of six determinations.
§ Average of three determinations.

Ammonium sulphamate was claimed by American workers[9] to reduce the 3:4-benzopyrene content of smoke by 80 per cent. when present at the level of 2 per cent. on the tobacco only and by 45 to 60 per cent. when present at the level of 4·25 per cent. on the paper only.

To test these claims, two separate experiments were carried out. Normal cigarettes made with untreated tobacco and untreated cigarette paper were used as controls and were compared with (*a*) cigarettes made from tobacco treated with 5 per cent. w/w of ammonium sulphamate and untreated cigarette paper and (*b*) cigarettes made from untreated tobacco and cigarette paper containing 4 per cent. w/w of ammonium sulphamate. The results are summarised in Table II.

These results show that treatment of tobacco with 5 per cent. w/w of ammonium sulphamate leads to an average reduction of about 60 per cent. in the amount of 3:4-benzopyrene in the smoke from cigarettes made from this tobacco and normal paper. On the other hand, treatment of the paper only with 4 per cent. w/w of ammonium sulphamate produces no detectable reduction in the 3:4-benzopyrene content of the smoke from cigarettes made from

TABLE II

EFFECT OF TREATMENT WITH AMMONIUM SULPHAMATE ON 3:4-BENZOPYRENE CONTENT OF CIGARETTE SMOKE

Experiment No.	Maximum 3:4-benzopyrene content of smoke from 500 g of cigarettes made from— untreated tobacco and untreated paper, μg	treated tobacco and untreated paper, μg	untreated tobacco and treated paper, μg
1	7·0	2·0	—
	3·0	1·5	—
	5·0	0·8	—
2	8·0	—	8·0
	8·0	—	8·0
	8·0	—	7·5

this paper and normal tobacco. This does not agree with the results reported by Alvord and Cardon[9] or by Lindsey and his co-workers.[10]

The levels of 3:4-benzopyrene reported by Alvord and Cardon[9] are much higher than those normally accepted for cigarette smoke, and the 3:4-benzopyrene content of the smoke from their cigarettes made with treated paper is much higher than those found by ourselves and other workers[11,12] in the smoke from normal cigarettes.

METHOD

3:4-Benzopyrene was determined in main-stream smoke by the method previously described.[6]

The various compounds tested were sprayed on to the tobacco as aqueous solutions, a rotating drum being used for this purpose in order to make distribution as even as possible. The solubility in water of each compound tested determined whether this spraying was carried out in one or more operations; if in more than one, the tobacco was dried after each spraying. After the final spraying the tobacco was conditioned to a suitable moisture content and made into cigarettes. At least three 500-g batches of cigarettes were smoked for each compound tested.

From each batch of tobacco used, an amount sufficient to make 2000 cigarettes was removed and sprayed as described above, but with distilled water. Cigarettes made from this tobacco were used as controls.

Cigarette paper impregnated with 4 per cent. w/w of ammonium sulphamate was obtained from Dr. C. Binns, of Robert Fletcher & Son Ltd., Greenfield, Yorkshire.

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NOTE—References 1 and 6 are to Parts II and I of this series, respectively.

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A Composite Procedure for the Analysis of Aluminium Bronze Alloys

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A procedure is described for determining copper, aluminium, iron, nickel, zinc, manganese, lead, silicon and tin in aluminium bronze alloys. Some of the methods used are conventional, but others are developments involving the techniques of spectrophotometry and polarography. The scheme is simple and, apart from the time needed for the electrolytic removal of copper, rapid. Results for three typical alloys are included.

The development of a composite scheme for analysing aluminium bronze alloys was undertaken because existing methods, often based on classical techniques, are lengthy and require a high degree of manipulative skill. The approximate composition of the alloys for which the scheme was designed was copper (80 per cent.), aluminium (10 per cent.), iron (5 per cent.), nickel (5 per cent.), manganese ($< 2{\cdot}5$ per cent.), zinc ($< 0{\cdot}5$ per cent.), lead ($< 0{\cdot}5$ per cent.), silicon (0·1 per cent.) and tin ($< 0{\cdot}5$ per cent.).

To minimise the time and effort needed, it was considered desirable to start from one weighing, to reduce the number of operations to the minimum and to make final measurements spectrophotometrically or polarographically.

Experimental

The existing procedure used at the beginning of this work involved an initial acid attack on the alloy, with subsequent removal of lead and silicon, as the insoluble sulphate and oxide, respectively. Copper was removed from the filtrate by electrolysis and was determined by the change in weight of the cathode. It was considered that the simplicity of such operations rendered them worthy of retention. On the grounds of accuracy, the gravimetric determinations of silicon and copper were retained, but an alternative procedure for determining lead was preferred. After solution of the lead sulphate in ammonium acetate solution, the method used is based on that described by Snyder,[1] who recommended that an appropriate aliquot of the solution be made alkaline to thymol blue by adding ammonia solution, that potassium cyanide solution be added and that the lead be extracted into a solution of dithizone in chloroform and then determined spectrophotometrically. However, in our application it was not necessary to add potassium cyanide solution, as interfering elements were absent.

It was intended that the electrolyte remaining after the removal of copper should be diluted to a fixed volume and that all subsequent determinations should be made on aliquots of this solution.

A search of the literature indicated that a complex formed between iron, ethylenediaminetetra-acetic acid (EDTA) and hydrogen peroxide had a suitable extinction coefficient. The principle was used by Ringbom, Siitonen and Saxén[2] for the spectrophotometric determination of iron in the presence of copper. The precipitation of aluminium, not considered by these workers, was prevented by prior addition of tartaric acid. The effect of other elements (such as nickel) that form coloured complexes with EDTA, but not with EDTA and peroxide, was counterbalanced by making measurements against an aliquot of the alloy solution to which EDTA, but not peroxide, had been added. Cobalt reacts in a manner similar to that of iron, but was absent from the alloys under consideration.

Reagents for determining nickel generally form coloured complexes having sensitivities in excess of that suitable for accurate measurement at the nickel content to be determined, so that the use of a difference technique was indicated. Nioxime[3] was preferred to dimethylglyoxime, as the presence of oxidising agents is not necessary for colour development. By making spectrophotometric measurements against a blank solution containing a slightly lower concentration of nickel than did the test solution, a satisfactory procedure was evolved; it was necessary to use Teepol as well as gum arabic to maintain the coloured complex in a dispersed state.

It was considered that, in view of the limitations attendant on colorimetric reagents for aluminium, a volumetric technique would be preferable; the procedure recommended by Sajó[4] was found to be applicable. An excess of EDTA solution was added to an aliquot of the electrolyte solution, and a copper solution was used to neutralise the excess. Sodium fluoride solution was then added to form a complex with aluminium more stable than the aluminium - EDTA complex, so liberating an amount of EDTA equivalent to the aluminium present. The liberated EDTA was then titrated with standard copper solution.

Dithizone,[5,6] or derivatives having similar properties, is a widely used reagent for zinc, although it lacks specificity. It was therefore necessary to consider possible interference from other elements present, notably iron, aluminium, manganese, nickel, tin and traces of copper. The first three of these elements were without effect, and it was possible to mask nickel by adding potassium cyanide solution, only the zinc complex, which is also formed, being decomposed by the subsequent addition of formaldehyde.[7] Since for accurate work it is necessary to determine the traces of copper remaining after electrolysis and since copper also reacts with dithizone, the extraction by and subsequent spectrophotometric determination of copper in a chloroform solution of neocuproine[8] was indicated at this stage. The presence of hydroxylamine hydrochloride as the necessary reducing agent was found to have no effect on stannic tin, which causes no interference. On these lines, satisfactory procedures for determining zinc and traces of copper were devised.

Manganese was determined by measuring the colour due to permanganic acid.

In view of the limited number of colorimetric procedures for tin, polarographic results were studied. Half-wave potentials reported in the literature[9] indicated that tin could be measured in hydrochloric acid solution and that interferences from the other elements present would be non-existent. The effects of changes in hydrochloric acid concentration and of the presence of sulphuric acid indicated that solution of the alloy in sulphuric acid and the use of hydroxylamine hydrochloride as reductant would afford suitable conditions for the determination. This was confirmed by subsequent measurements on sample solutions.

Method

Procedure—

Dissolve 2·000 g of sample in 60 ml of a mixture containing 20 per cent. v/v each of sulphuric and nitric acids, and heat until nitrous fumes have been expelled. Add 10 ml of hydrochloric acid, sp.gr. 1·16, and evaporate until fumes of sulphur trioxide are evolved. Cool, add 100 ml of water, and boil until all soluble salts have dissolved. Set aside for 1 hour, filter through a pad of filter-paper pulp, and wash the residue with sulphuric acid (5 per cent. v/v) until free from copper salts. Reserve the filtrate for electrolysis.

Determination of lead—Dissolve the lead sulphate by pouring a hot solution of ammonium acetate (approximately 30 per cent. w/v) through the filter, and dilute to 250 ml. To a 5-ml aliquot of this solution add 20 ml of ammonium acetate solution and 10 ml of ammonia solution (sp.gr. 0·880). Dilute to approximately 75 ml, and extract with 25 ml of a 0·01 per cent. solution of dithizone in carbon tetrachloride. With a suitable spectrophotometer, measure the optical density of this solution at 535 mμ in a 1-cm cell against a blank prepared by similar extraction of the reagents used. Determine the lead content of the alloy by reference to a calibration graph plotted from measurements made on extracts of pure lead solutions.

Determination of silicon—Transfer the pad of filter-paper containing silica to a platinum crucible, char at 400° C, and then heat at 1000° C for several minutes. Allow to cool in a desiccator, record the weight of the residue, and calculate the percentage of silicon in the alloy. (It has been found unnecessary to correct for impurities in the silica by the customary treatment with hydrofluoric acid, the error arising from this omission being less than 0·01 per cent.)

Determination of copper—Dilute the reserved filtrate to approximately 300 ml, and add 2 g of ammonium nitrate. Insert a weighed platinum-gauze cathode and a platinum-wire anode, and electrolyse the solution at a current density of 2·5 amps per sq. dm. When the solution is almost colourless, add 1 to 2 g of urea, and continue electrolysis until deposition of copper is complete. Wash the cathode with water and then with ethanol, dry, and weigh to determine the copper content of the alloy.

Dilute the electrolyte to 500 ml, and use this solution for the determination of the remaining elements.

Determination of iron—Withdraw two 10-ml aliquots of the electrolyte solution. To each of these add successively 1 ml of tartaric acid solution (10 per cent. w/v), 10 ml of EDTA solution (0·05 *M*) and 10 ml of ammonia solution (sp.gr. 0·880). To one mixture add 4 ml of 30-volume hydrogen peroxide, and dilute both solutions to 100 ml. With a suitable spectrophotometer, measure the optical density of the highly coloured solution, with the other solution as compensating blank, at 520 mμ in a 4-cm cell. Determine the iron content of the alloy by reference to a calibration graph plotted from measurements made on solutions prepared from suitable amounts of pure iron.

Determination of nickel—To a 10-ml aliquot of the electrolyte solution add 10 ml of buffer solution (250 g of citric acid and 350 ml of ammonia solution, sp.gr. 0·880, diluted to 500 ml) and 5 ml of gum arabic solution (10 per cent. w/v). Dilute to approximately 90 ml, add 1 ml of Teepol and 4 ml of nioxime solution (0·8 per cent. in ethanol - water mixture containing 10 per cent. of ethanol), and dilute to 100 ml. Prepare a blank by similarly treating a solution containing 2·0 mg of iron and 1·600 mg of nickel, and measure the optical density of the test solution against the blank in a 1-cm cell at 550 mμ with a suitable spectrophotometer. Determine the nickel content of the alloy by reference to a calibration graph plotted from measurements made on solutions containing 2 mg of pure iron and 1·6 to 2·4 mg of pure nickel (corresponding to 4 to 6 per cent. of nickel in the alloy).

Determination of aluminium—To a 50-ml aliquot of the electrolyte solution add 25 ml of EDTA solution (0·05 *M*), neutralise to methyl red with ammonia solution, sp.gr. 0·880, and add 10 ml of a buffer solution prepared by dissolving 15 g of sodium acetate trihydrate in 500 ml of water, adding 5 ml of glacial acetic acid and diluting to 1 litre. Boil for 3 minutes, cool, add approximately 20 drops of 1-(2-pyridylazo)-2-naphthol solution (0·05 per cent. w/v in ethanol), and titrate with 0·1 *N* copper sulphate until the colour of the solution just changes to violet. Add 30 ml of a saturated solution of sodium fluoride, boil for 3 minutes, cool, and titrate with 0·1 *N* copper sulphate until the solution is blue. Calculate the aluminium content of the alloy from the aluminium equivalents of the copper sulphate and EDTA solutions (obtained from similar titrations of standard aluminium solutions).

Determination of zinc and traces of copper—Withdraw a 25-ml aliquot of the electrolyte solution, and dilute to 100 ml. To 5 ml of this solution add 10 ml of buffer solution (100 g of citric acid *plus* sufficient ammonia solution, sp.gr. 0·880, to bring the pH of the mixture to 8·70 ± 0·2) and 2 ml of hydroxylamine hydrochloride solution (10 per cent. w/v). Extract with a mixture of 2 ml of neocuproine solution (0·1 per cent. in ethanol) and 10 ml of chloroform and then with a mixture of 1 ml of neocuproine solution and 5 ml of chloroform. Reserve the aqueous layer for the determination of zinc.

Add 2 ml of ethanol to the combined extracts, and dilute to 25 ml with chloroform. Measure the optical density of this solution against pure chloroform at 455 mμ in a 1-cm cell, and determine the concentration of copper by reference to a calibration graph plotted from measurements made on similar extracts of solutions of pure copper (adjusted to the correct pH value). To the reserved aqueous layer add 5 ml of potassium cyanide solution (1 per cent. w/v) and 5 ml of formaldehyde solution (4 per cent. v/v), and adjust the pH to 8·40 ± 0·05 by adding dilute ammonia solution or dilute hydrochloric acid. Extract the zinc by shaking vigorously for 1 minute with 20 ml of dithizone solution (0·01 per cent. in carbon tetrachloride), dilute the extract to 25 ml, and measure the optical density of this solution at 535 mμ in a 1-cm cell against a blank prepared by extracting the various reagents used. Calculate the zinc content by reference to a calibration graph plotted from measurements made on similarly treated solutions of pure zinc.

Determination of manganese—To a 25-ml aliquot of the electrolyte solution add 15 ml of potassium periodate solution (0·75 per cent. w/v), and boil gently for 5 minutes. Cool, dilute to 100 ml, and withdraw two 25-ml aliquots. To one aliquot add 1 ml of water and to the other add 1 ml of sodium nitrite solution (1 per cent. w/v). Measure the optical density of the coloured solution against the reduced solution at 526 mμ in a 1-cm cell, and calculate the manganese content of the alloy by reference to a calibration graph plotted from measurements made on standard solutions of potassium permanganate.

Determination of tin—Evaporate a 5-ml aliquot of the electrolyte solution until fumes of sulphur trioxide are evolved. Add 5 ml of water, 2 ml of hydroxylamine hydrochloride solution (10 per cent. w/v) and 0·5 ml of gelatin solution (0·5 per cent. w/v). Dilute to

25 ml, remove dissolved oxygen by passing nitrogen through the solution for several minutes, and record a polarogram from −0·3 to −0·8 volt against a mercury-pool anode. Calibrate by making measurements on portions of a standard tin solution.

Discussion of the method

The most time-consuming stage of the proposed method is the removal of copper by electrolysis, but this is unimportant, as lead and silicon can be determined in the interim. The time taken to analyse a single sample is approximately 3½ hours.

Table I

Comparison of results by proposed and conventional procedures

Element	Element found in alloy A by—		Element found in alloy B by—		Element found in alloy C by—	
	proposed procedure, %	conventional procedure, %	proposed procedure, %	conventional procedure, %	proposed procedure, %	conventional procedure, %
Lead	0·43, 0·42 0·41, 0·41	0·41, 0·39 —, —	0·10, 0·11 0·11, 0·09	0·08, 0·09 —, —	0·09, 0·07 0·08, 0·09	0·08, 0·07 —, —
Silicon	Conventional	0·10, 0·10 0·10, 0·11	Conventional	0·15, 0·14 0·15, 0·15	Conventional	0·03, 0·03 0·02, 0·03
Copper	Conventional	78·12, 78·14 78·17, 78·25	Conventional	78·76, 78·66 78·71, 78·68	Conventional	84·33, 84·27 84·22, 84·35
Iron	4·00, 4·01 4·00, 4·00	4·06, 4·02 —, —	3·85, 3·85 3·85, 3·85	3·90, 3·90 —, —	3·09, 3·09 3·07, 3·10	3·09, 3·13 —, —
Nickel	5·22, 5·24 5·23, 5·23	5·25, 5·28 —, —	5·03, 5·07 5·03, 5·04	5·05, 5·09 —, —	4·96, 4·98 4·97, 4·98	4·98, 4·93 —, —
Aluminium	10·63, 10·63 10·63, 10·61	10·56, 10·47 —, —	10·21, 10·22 10·20, 10·24	10·16, 10·21 —, —	6·82, 6·87 6·84, 6·87	6·86, 6·92 —. —
Copper (traces)	0·025, 0·030 0·020, 0·020	Not determined	0·030, 0·035 0·025, 0·030	Not determined	0·020, 0·020 0·017, 0·025	Not determined
Zinc	0·19, 0·20 0·20, 0·21	0·18, 0·20 —, —	0·095, 0·11 0·10, 0·11	0·09, 0·08 —, —	0·23, 0·23 0·24, 0·24	0·23, 0·26 —, —
Manganese	Conventional	1·05, 1·07 1·05, 1·06	Conventional	1·67, 1·67 1·66, 1·67	Conventional	0·17, 0·19 0·18, 0·17
Tin	0·10, 0·12 0·11, 0·12	0·10, 0·10 —, —	0·23, 0·21 0·24, 0·23	0·19, 0·21 —, —	0·18, 0·18 0·16, 0·17	0·16, 0·15 —, —

In Table I results by the proposed method are compared with those found by more conventional methods. For example, iron was separated as hydroxide and was determined by adding an excess of potassium iodide solution and then titrating with sodium thiosulphate solution; nickel was separated as its complex with dimethylglyoxime and was determined by titration with potassium cyanide and silver nitrate solutions; aluminium was separated as its complex with 8-hydroxyquinoline and was determined by titration with potassium bromate - bromide mixture and sodium thiosulphate solution; lead was determined by an alternative dithizone method,[10] and zinc was determined polarographically after separation by ion exchange.

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The Determination of Copper in Lead and Lead Cable-sheathing Alloys

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A method is described for determining copper in lead and lead cable-sheathing alloys. The sample is dissolved in a hydrogen peroxide - acetic acid mixture, converted to a chloride solution, and copper is extracted with diethylammonium diethyldithiocarbamate in carbon tetrachloride. After destruction of the organic ligand, the pH of the solution is adjusted to between 8 and 9·5 and the blue copper - bis*cyclo*hexanone oxalyldihydrazone complex is formed. The optical density of the blue complex is measured spectrophotometrically at 595 mμ, and the copper content is read from a calibration graph. The usual amounts of antimony, tin, cadmium, bismuth, tellurium, silver, zinc and arsenic encountered in the alloys do not interfere.

Copper has been determined absorptiometrically in a number of materials by means of its diethyldithiocarbamate complex. It is usual to extract the copper into an organic solvent, such as chloroform, with diethylammonium diethyldithiocarbamate, when other metals that react under the conditions employed also pass into the organic phase and often interfere with the subsequent optical-density measurements. In order to separate quantitatively copper from lead it is necessary to perform the extraction from a solution 4 to 6 *N* in hydrochloric acid.[1] In so doing, metals such as bismuth present in the alloy are removed at the same time, and a correction has to be applied to the optical-density readings to allow for any interference. If, alternatively, extraction is carried out from ammoniacal citrate solution, bismuth is again removed with the copper.[2] Bismuth may be present in lead and lead cable-sheathing alloys in significant amounts, and consequently it was apparent that a modified or alternative absorptiometric method was desirable for use with these materials.

A procedure for determining copper in certain high-alloy steels has been developed in these laboratories.[3] Copper is separated as its dithizonate in a carbon tetrachloride solution and, after destruction of the organic ligand, the determination is completed by measuring absorptiometrically the blue copper - bis*cyclo*hexanone oxalydihydrazone complex. Successful attempts have been made to develop a procedure for determining copper in lead and lead cable-sheathing alloys by means of this complex.

Experimental

Initially, tests were carried out to check the use of bis*cyclo*hexanone oxalyldihydrazone for determining copper directly in a buffered sample solution by a procedure similar to that used by Haywood and Sutcliffe[4] for determining copper in ferrous materials. Low results were obtained owing to significant fading of the blue colour, especially with higher concentrations of copper, as subsequent formation of a blue precipitate containing lead and copper took place. The approximate degree of fading for a solution of 250 mg of lead in 100 ml ranged from negligible at 0·01 per cent. of copper to 30 per cent. in 30 minutes for 0·1 per cent. of copper. In view of these findings it was obvious that preliminary separation was necessary.

Experiments were carried out in which an extraction with dithizone was preferred to a classical chloride separation. Sulphuric acid could not be used to form the extraction

solution, but it was shown that copper dithizonate could be quantitatively removed from a solution 0·25 *N* in nitric acid; it was therefore a simple matter to determine copper in plain lead and alloys B and D by a method similar to that used for copper in steel.[3] Alloy E (containing 0·4 per cent. of tin and 0·2 per cent. of antimony) could not be treated in this way owing to possible co-precipitation errors that would accompany the separation of metastannic acid from dilute solutions of nitric acid. A hydrogen peroxide - acetic acid mixture[5] was used alternatively as solvent for this alloy; citric acid was used to prevent precipitation of the lead, excess of peroxide was destroyed by boiling, and the acidity was adjusted with trichloroacetic acid. Copper was then removed and determined as previously, except that a reagent blank had to be accounted for. Good results were obtained by both procedures.

In spite of the success, it was considered desirable to provide one single procedure that could be used for the analysis of plain lead and all three cable-sheathing alloys. Further modifications of the dithizone separation were ignored, and the alternative dithiocarbamate method of removing the copper[1] was arbitrarily applied. Concentrated hydrochloric acid does not present a rapid method for dissolving lead alloys, and consequently hydrogen peroxide and acetic acid, as recommended by Hamilton,[5] were used for this purpose. Excess of peroxide was destroyed by evaporating the solution just to dryness, and 6 *N* hydrochloric acid was then added to form the extraction solution. Copper was separated by means of a solution of diethylammonium diethyldithiocarbamate in carbon tetrachloride. The complex was destroyed with an oxidising acid mixture before proceeding with the formation of the bis*cyclo*hexanone oxalyldihydrazone complex.

Method

Reagents—

Hydrogen peroxide, 100 *volume*.

Acetic acid, glacial.

Hydrochloric acid, 6 N.

Diethylammonium diethyldithiocarbamate solution—Prepare a 1 per cent. solution of diethylammonium diethyldithiocarbamate in carbon tetrachloride.

Carbon tetrachloride.

Oxidising acid mixture—Mix 1 volume of sulphuric acid, sp.gr. 1·84, 1 volume of nitric acid, sp.gr. 1·42, and 2 volumes of perchloric acid, sp.gr. 1·70.

Citric acid solution—Prepare a 25 per cent. w/v solution of citric acid in water.

Ammonia solution, sp.gr. 0·880.

α-Naphtholphthalein indicator solution—Prepare a 0·1 per cent. solution of the indicator in 50 per cent. aqueous ethanol.

*Bis*cyclo*hexanone oxalyldihydrazone solution*—Prepare a 0·5 per cent. solution of the reagent in 50 per cent. aqueous ethanol.

Standard copper solution—Prepare a solution containing 25 μg per ml by dissolving spectrographically pure copper in a small volume of diluted nitric acid (1 + 1) and diluting to the required volume.

Procedure—

Dissolve 0·25 g of sample in a mixture of 5 ml of water, 5 ml of 100-volume hydrogen peroxide and 2·5 ml of glacial acetic acid in a 125-ml conical beaker covered with a watch-glass. Boil the solution gently, evaporating almost to dryness, and remove from the source of heat immediately the final vigorous effervescence has taken place. Wash the watch-glass and sides of the beaker with 6 *N* hydrochloric acid. Add about 40 ml of this acid, and warm the beaker to dissolve the precipitated lead chloride. Allow the solution to boil, and then cool to room temperature. Transfer the solution to a 100-ml separating funnel with 6 *N* hydrochloric acid, and extract successively with three 10-ml portions of diethylammonium diethyldithiocarbamate solution and finally with 10 ml of carbon tetrachloride. (Metals such as bismuth are removed with the copper, and to ensure that the extraction of copper is carried out quantitatively these metals must be completely removed; consequently it may be necessary to use more than three portions of the extraction solution.) Combine the extracts in a 125-ml conical beaker, and add 5 ml of oxidising acid mixture. Cover with a watch-glass, and heat gently to remove carbon tetrachloride and then strongly to destroy the remaining organic matter. Ensure that the solution is evaporated until fumes are evolved and that the solution is completely colourless. (Failure to destroy all the organic

matter can be a cause of high results.) Cool to room temperature, wash the watch-glass and sides of the beaker with distilled water, diluting to about 20 ml, and add 5 ml of 25 per cent. w/v citric acid solution. Adjust the pH to between 8·0 and 9·5 with ammonia solution, sp.gr. 0·880 (see Note), and transfer the solution to a 100-ml calibrated flask. Place the flask in a thermostatically controlled water bath at 20° C, and add 5 ml of 0·5 per cent. bis*cyclo*hexanone oxalyldihydrazone solution. (A temperature of 20° C was chosen arbitrarily for the dilution of all solutions to a standard volume in order to avoid the difficulties encountered at lower temperatures; *e.g.*, below 15° C formation of the copper complex is sometimes delayed.) Shake the flask, allow the colour to develop for 5 minutes, and adjust the volume to 100 ml with distilled water at 20° C.

Shake well, and measure the optical density of the solution with a spectrophotometer at 595 mμ in a 1-cm cell with the instrument adjusted against a reagent blank solution. Read the copper content of the alloy under test from a linear calibration graph plotted from a range of standard copper solutions that have been taken through the procedure.

NOTE—Use narrow-range indicator papers in strips of about $\frac{1}{16}$th inch to adjust the pH. Carry out a check by placing 1 drop of test solution and 1 drop of α-naphtholphthalein indicator solution about 0·5 cm apart on a filter-paper. As the drops merge, a distinct blue colour is observed when the pH of the test solution is in the correct range. Any indeterminate colours or colours produced when the spots approach each other but do not merge must be ignored. The procedure was devised to overcome the effects of ammonia evolved from the buffered solutions, which should be stoppered if set aside for any length of time.

DISCUSSION OF THE METHOD

The most useful range of copper concentrations in the final solutions is approximately 25 to 250 μg per 100 ml, *i.e.*, 0·01 to 0·10 per cent. of copper in a 250-mg sample. It is, however, a simple matter to extend this range, over which Beer's law is obeyed, by adjusting the weight of sample. If a larger sample is used, care should be taken to ensure that sufficient 6 *N* hydrochloric acid is present to retain the lead chloride in solution before the extraction of copper is carried out. For higher concentrations of copper, an aliquot of the sample solution may be conveniently used to carry out the analysis.

The effect of other elements present in lead cable-sheathing alloys on the determination of copper was examined; 1·2 per cent. of antimony, 0·8 per cent. of tin, 0·5 per cent. of cadmium, 0·1 per cent. of bismuth and 0·01 per cent. each of tellurium, silver, zinc and arsenic did not interfere. These are the maximum concentrations likely to be encountered in the analysis of these alloys, but there is no indication that higher amounts would cause any interference.

RESULTS

Recoveries of known amounts of copper from synthetic alloys prepared from solutions are shown in Table I. Optical-density measurements were made with a Unicam SP500 spectrophotometer. The composition of the lead cable-sheathing alloys included the approximate maximum amounts of the impurities and alloying elements listed in the British Standard,[6] *viz.*, 0·005 per cent. each of silver and tellurium, 0·05 per cent. of bismuth, 0·002 per cent.

TABLE I

RECOVERY OF COPPER FROM LEAD CABLE-SHEATHING ALLOYS BY THE PROPOSED METHOD

Alloy B		Alloy D		Alloy E	
Copper present, %	Copper found, %	Copper present, %	Copper found, %	Copper present, %	Copper found, %
0·010	0·009	0·010	0·011	0·010	0·011
0·030	0·030	0·030	0·029	0·030	0·030
0·050	0·052	0·050	0·049	0·050	0·051
0·070	0·070	0·070	0·069	0·070	0·070
0·080	0·079	0·080	0·079	0·080	0·080
0·100	0·100	0·100	0·099	0·100	0·100
0·010	0·010	0·010	0·011	0·010	0·010
0·050	0·049	0·050	0·049	0·050	0·050
0·100	0·099	0·100	0·098	0·100	0·100

of zinc and 0·01 per cent. of other elements (including cadmium in alloys B and E). Alloy B contained 0·85 per cent. of antimony, alloy D contained 0·5 per cent. of antimony and 0·25 per cent. of cadmium and alloy E contained 0·4 per cent. of tin and 0·2 per cent. of antimony.

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Stoicheiometry of Titration of Calcium, Magnesium and Manganese at Low Concentration with EDTA, with the Metal Indicators Murexide and Eriochrome Black T

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The titrations of calcium, magnesium and manganese to determined colours of the indicators murexide and Eriochrome black T were compared with values calculated from the theoretical formulae. The results show that the titration of calcium at higher α values is stoicheiometric within the accuracy of the titration. For the titration of magnesium at room temperature there is a difference between the calculated and the observed values. As this error is greatly reduced by titration at elevated temperature, it is thought to be due to a slow rate of reaction.

The titration results for manganese with Eriochrome black T as indicator were in good agreement with the theoretical. However, a small discrepancy between titrations at room and elevated temperatures also indicated a slow rate of reaction.

In the presence of calcium and magnesium, manganese can be calculated from the difference between the titre for calcium, magnesium and manganese together with EDTA, Eriochrome black T being used as indicator, and the titre for calcium *plus* magnesium after manganese has been removed with sodium diethyldithiocarbamate and carbon tetrachloride. The choice of organic solvent for the extraction is discussed.

Complexometric titrations of calcium and magnesium with EDTA (disodium ethylenediaminetetra-acetate) have been used increasingly in the past 10 years, and the volume of literature on this subject is continually growing.[1,2,3,4,5] Blaedel and Knight[6] have studied the stoicheiometry of the titrations of calcium and magnesium by a high-frequency technique; they used metal concentrations of approximately $2 \times 10^{-3}\,M$ and found that the titration was stoicheiometric within relative errors of approximately 0·05 per cent. for calcium and 0·1 per cent. for magnesium. Ringbom and Vänninen[7] and Fortuin, Karsten and Kies[8] have developed the theory for photo-electric complex-formation titration with metal indicators. A similar theoretical treatment has been applied to visual titration.[9] The colour of the end-point in a visual titration is determined before the titration is carried out. The colour at the equivalence-point is, however, influenced by the stability constants of the complexes and the concentrations of metal and indicator; this can lead to a titration error. This error

can be calculated from the law of mass action. An error can arise if the rate of reaction is slow; it can, however, be reduced by increasing the titration temperature.

In this paper, formulae are developed for complexometric titration to a pre-determined end-point colour, and calculations are compared with results for titrations of calcium, magnesium and manganese.

Theoretical Treatment

Symbols—

C_M = total metal concentration.
$[M]$ = concentration of metal ion.
$[MY]$ = concentration of metal-containing titrant (EDTA).
$[MI]$, $[MI_2]$ = concentrations of the metal-containing indicator.
C_Y = total concentration of titrant (EDTA).
$[Y]$ = concentration of metal-free titrant.
C_I = total concentration of indicator.
$[I]$ = concentration of metal-free indicator.
$[A]$ = concentration of anions, *e.g.*, hydroxyl.
$[MA]$ = concentration of metal-containing anions.

$$\alpha = \frac{[I]}{C_I}\text{: indicator change ratio} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$K_{MY} = \frac{[MY]}{[M]\,[Y]}\text{: stability constant of MY} \quad .. \quad .. \quad .. \quad (2)$$

$$K_{MI} = \frac{[MI]}{[M]\,[I]}\text{: stability constant of MI} \quad .. \quad .. \quad .. \quad (3)$$

$$K_{MI_2} = \frac{[MI_2]}{[M]\,[I]^2}\text{: stability constant of } MI_2 \quad .. \quad .. \quad .. \quad (4)$$

$$K_{MA} = \frac{[MA]}{[M]\,[A]}\text{: stability constant of MA} \quad .. \quad .. \quad .. \quad (5)$$

Derivation of a General Expression for the Titration—

The total concentration of EDTA in the titrated solution can be expressed by—

$$C_Y = [MY] + [Y] \quad .. \quad .. \quad .. \quad .. \quad (6)$$

The total metal concentration will be—

$$C_M = [MY] + [MI] + [MI_2] + [MA] + [M] \quad .. \quad .. \quad .. \quad (7)$$

and the total indicator concentration will be—

$$C_I = 2[MI_2] + [MI] + [I] \quad .. \quad .. \quad .. \quad .. \quad (8)$$

From equations (1) to (8) an expression for the total concentration of EDTA is obtained by substitution—

$$C_Y = \left(1 + \frac{K_{MI}\alpha + 2K_{MI_2}C_I\alpha^2}{K_{MY}(1-\alpha)}\right)C_M -$$

$$\left(\frac{1-\alpha}{K_{MI}\alpha + 2K_{MI_2}C_I\alpha^2} + \frac{1}{K_{MY}}\right)\left(1 + K_{MI}C_I\alpha + K_{MI_2}C_I^2\alpha^2 + K_{MA}[A]\right) \quad .. \quad (9)$$

(The term inside the brackets before C_M is the theoretical value of the slope and the product of the terms after the minus sign is the theoretical value of the intercept.)

The stability constants in equation (9) may be designated as "apparent" in accordance with Schwarzenbach's[1] "*scheinbare Indikatorkonstanten*," because they depend on the pH of the solution.

From equation (9) it can be seen that, when two metal - indicator complexes exist, *e.g.*, zinc Eriochrome black T,[1] the slope depends not only on the colour-change ratio, but also on the indicator concentration. The intercept, however, is always a function of the concentration of the indicator. The deviation of the slope from unity increases with increasing

values of α and increasing ratios of the stability constants of the metal - indicator complexes to the stability constant of the metal - EDTA complex. The intercept depends on this ratio as well as the absolute value of the stability constants. The numerical value of the intercept increases with increase in this ratio and with decrease in the values of the stability constants.

The effect of other ions bound to the metal is to increase the value of the intercept.

Colour comparisons

The colours of murexide and, especially, Eriochrome black T are not particularly stable at high pH, and it is therefore desirable to use coloured reference solutions to determine the exact end-points of the titrations.

Reference colour standards for titration of calcium—

Two different colours between the red of calcium murexide and the violet of calcium-free murexide were chosen. These colours corresponded to the ratio $\alpha = \frac{[I]}{C_I} = 0{\cdot}85$ and $\alpha = 0{\cdot}97$, where C_I is the concentration of murexide used in the titrations ($C_I = 2{\cdot}8 \times 10^{-5}\ M$). These colour determinations were carried out in test-tubes with a comparator. Artificial standards identical to the two chosen colours were prepared from Gentian violet and methyl red. Mixtures of 0·6 ml of 0·01 per cent. Gentian violet with 0·25 or 0·30 ml of 0·01 per cent. methyl red in 100 ml of water acidified to about pH 3 with sulphuric acid gave the desired colours.

Reference colour standards for titrations of magnesium and manganese—

Two different colours were chosen between the red of metal Eriochrome black T and the blue of metal-free Eriochrome black T. The colours chosen corresponded to the ratios $\alpha = 0{\cdot}8$ and $\alpha = 0{\cdot}9$. The indicator concentration, C_I, was $1{\cdot}8 \times 10^{-6}\ M$. The comparator method was used and the results were controlled with a Beckman DU spectrophotometer at 6150 A, *i.e.*, the wavelength of maximum absorption of the metal-free dye.

Artificial standards were prepared from methylene blue and methyl red. Mixtures of 0·70 ml of 0·01 per cent. methylene blue with 0·10 or 0·14 ml of 0·01 per cent. methyl red in 100 ml of water acidified to about pH 3 with sulphuric acid gave the desired colours.

Titration of calcium

Reagents—

Standard EDTA solution—A 0·003000 *M* solution of EDTA was prepared from the dihydrate as a primary standard after purification and drying by Blaedel and Knight's method.[10]

A similarly prepared solution of EDTA was checked against a primary zinc standard, prepared from metallic zinc (Merck). The results from ten titrations gave an assay value of 100·074 per cent. of EDTA, the standard deviation of a single titration being 0·11 per cent.

Standard calcium solution—A 0·01869 *M* solution of calcium chloride was prepared from calcium carbonate as a primary standard. From this stock solution, a 0·001495 *M* solution was prepared by dilution. The calcium carbonate was prepared from calcium acetate by dissolving 5 g of calcium acetate (Baker's Analyzed) in 500 ml of water acidified with 1 ml of concentrated acetic acid. Calcium was precipitated as oxalate by adding to the hot calcium solution 50 ml of a warm solution of 5 g of ammonium oxalate. The coarse-grained precipitate was spun in a centrifuge and washed twice with water. After it had been dried at low temperature, the precipitate was ignited in an electric furnace at 500° ± 25° C to constant weight.[11] The residue was moistened with 1 ml of a saturated solution of ammonium carbonate, dried at 120° C, and weighed. The moistening with ammonium carbonate solution and drying were repeated; the residue, however, did not change in weight.

A standard calcium solution was also prepared from calcium carbonate (Merck) as a primary standard. Within an error of ±0·1 per cent. no difference was found by titration with EDTA between this and the specially prepared primary standard.

Potassium hydroxide solution, 1 M.

Murexide indicator, powdered—A 0·2-g portion of murexide (Merck) mixed with 50 g of sodium chloride and ground to a fine powder.

Gentian violet solution, 0·01 *per cent.*

Methyl red solution, 0·01 *per cent.*

Water—Water distilled from Pyrex-glass apparatus and passed through a column of Dowex 50 ion-exchange resin.

PROCEDURE—

To 1-, 2-, 5-, 10- and 20-ml aliquots of 0·001495 *M* calcium solution in separate 200-ml flasks were added 2 ml of potassium hydroxide solution; each solution was then diluted so that the total volume after titration would be approximately 50 ml. Separate 50-mg portions of murexide indicator powder were added to each, and the calcium was titrated with standard EDTA solution to the same colour as that of the reference colour standard chosen.

RESULTS—

The results of five sets of quintuplicate determinations expressed in millimoles per litre at the end-point of the titration and treated statistically by the method of least squares are shown in Table I. The slope and intercept can be calculated from equation (9) by using an indicator concentration, C_I, of $2{\cdot}8 \times 10^{-5}$ *M* and apparent stability constants at pH 12, $K_{CaY} = 10^{10.70}$, $K_{CaI} = 10^{5}$ and $K_{CaOH} = 25$, as given by Schwarzenbach.[1] Agreement between the observed and theoretical values is good. Although the end-point is not exactly at the equivalence-point, it may be concluded that at α values above 0·97 the titration is stoicheiometric within the accuracy of the titration.

Titrations of calcium performed in presence of hydroxylamine hydrochloride and potassium cyanide gave similar results.

TABLE I

CALCULATED AND OBSERVED VALUES FOR THE TITRATION OF CALCIUM

	Value of α at the end-point	Slope	Intercept, millimolar	Standard deviation, millimolar	Number of determinations
Calculated	0·85	1·0000	−0·0042	—	—
	0·97	1·0000	−0·0008	—	—
Observed	0·85	0·9963 ± 0·0006	−0·0029	0·0007	25
	0·97	0·9988 ± 0·0005	−0·0005	0·0006	25

TITRATION OF MAGNESIUM

REAGENTS—

Standard EDTA solution—As used for the titration of calcium.

Standard magnesium solution—A 0·02563 *M* solution of magnesium was prepared from analytical-reagent grade magnesium sulphate heptahydrate (Merck) after dehydration by Blaedel and Knight's method.[6] From this stock solution a 0·001268 *M* solution was prepared by dilution.

Buffer solution, pH 10—A solution of 67·5 g of ammonium chloride in 570 ml of concentrated ammonia solution was diluted to 1 litre with water.

Methylene blue solution, 0·01 *per cent.*

Potassium cyanide solution, 2 *per cent.*

Hydroxylamine hydrochloride solution, 10 *per cent.*

Eriochrome black T solution—A solution of 0·05 g of Eriochrome black T (British Drug Houses Ltd.) and 2 g of hydroxylamine hydrochloride in 100 ml of methanol.

The purity of the Eriochrome black T was determined by Diehl and Lindstrom's method.[12] The absorbancy at pH 10 of the dye dried in a vacuum desiccator over phosphorus pentoxide was measured with a Beckman DU spectrophotometer at 6150 A; the value of $E^{1\%}_{1cm}$ was 278. The $E^{1\%}_{1cm}$ value for the purified dye deduced from Diehl and Lindstrom's observations (calculated as sodium salt from the molar extinction coefficient of 32,300) was 701, which gives a purity for the British Drug Houses' preparation of 40 per cent. The accuracy of this figure is not essential.

PROCEDURE—

To 1-, 2-, 5-, 10- and 20-ml aliquots of 0·001268 *M* magnesium solution in separate 200-ml flasks were added 1 ml of hydroxylamine hydrochloride solution, 5 ml of buffer solution

and 0·5 ml of potassium cyanide solution; each solution was then diluted so that the total volume after titration would be approximately 50 ml. To each, 0·2 ml of indicator solution was added, and magnesium was titrated with standard EDTA solution to the same colour as that of the reference colour standard.

RESULTS—

The results, treated statistically in the same way as those for calcium, are shown in Table II. The slope and intercept were calculated by using an indicator concentration, C_I, of 0·0018 mM and apparent stability constants at pH 10, $K_{MgY} = 10^{8.20}$, $K_{MgI} = 10^{5.44}$ and $K_{MgOH} = 10^{2.58}$, as given by Schwarzenbach.[1]

TABLE II

CALCULATED AND OBSERVED VALUES FOR THE TITRATION OF MAGNESIUM

	Value of α at the end-point	Temperature, °C	Slope	Intercept, millimolar	Standard deviation, millimolar	Number of determinations
Calculated	0·8	—	1·0070	−0·0004	—	—
	0·9	—	1·0157	−0·0002	—	—
Observed	0·8	20	1·013 ± 0·0008	0·0007	0·0006	20
	0·9	20	1·028 ± 0·0011	0·0030	0·0009	20
Observed	0·8	60	1·007 ± 0·0010	−0·0012	0·0008	20
	0·9	60	1·015 ± 0·0013	0·0011	0·0007	10

It can be seen from Table II that both the slope and intercept are higher than those calculated. As this might be caused by slow rate of reaction, the titrations were repeated at a higher temperature, as recommended by de Sousa.[13] The temperature chosen was 60° C; results for these titrations are also shown in Table II and indicate better agreement with the calculated values. At higher temperature the colour change is less sharp than at room temperature, but for both the standard deviation and mean error are small, so that the reproducibility of the titrations is satisfactory. A separately standardised solution of EDTA is, however, required for determining magnesium at these concentrations, since the end-point differs significantly from the equivalence-point. The use of colour comparison is imperative, a fact also pointed out by Connors.[14] A suitable end-point colour is that corresponding to $\alpha = 0{\cdot}8$, which agrees well with bluish purple-blue, 2·5 (PB), given in "Munsell Book of Color."[15]

Potassium cyanide and hydroxylamine hydrochloride are not necessary when titrating pure solutions; similar results were obtained without these reagents. They have, however, a marked stabilising effect on the indicator, preventing a fast fading of the colour. As they had no visible influence on the colour change, they were included, especially as they are essential when impure solutions are to be titrated.

TITRATION OF MANGANESE

REAGENTS—

Standard EDTA solution—As used for the titration of calcium.

Standard manganese solution—A 0·01593 M solution of manganese was prepared from analytical-reagent grade manganese sulphate tetrahydrate (Merck) after drying at 600° C. From this stock solution a 0·0007965 M solution was prepared by dilution.

PROCEDURE—

Various aliquots of 0·0007965 M manganese solution were titrated with standard EDTA solution. The titrations were carried out at 20° and 60° C by the same procedure as used for magnesium. Hydroxylamine hydrochloride in the solution prevented oxidation of the manganese. The buffer solution should, however, be added to the solution immediately before titration, as longer standing causes results that are too low, especially at higher manganese concentrations.

Owing to its complexing effect on manganese, addition of triethanolamine before the titration, as recommended by Přibil,[16] gives a less sharp colour change in this concentration range; it also increases, in accordance with equation (9), the value of the intercept.

The presence of potassium cyanide is not necessary when titrations are carried out with

pure solutions, and its exclusion had no influence on the results or colour change at $\alpha = 0{\cdot}8$ and 0·9. It has, however, a favourable effect on the stability of the indicator, probably by masking traces of the heavy metals always present, even in analytical-reagent grade materials. Potassium cyanide is therefore included, as its presence is necessary when impure solutions have to be titrated.

RESULTS—

The results, calculated in the same way as those for calcium and magnesium, are shown in Table III. The stability constant of the manganese - indicator complex is not known, but the stability constant of the complex of manganese with EDTA is high; $K_{MnY} = 10^{13{\cdot}79}$. The sharpness of the end-point colour change indicates a favourable ratio between this and the constant of the manganese - indicator complex. Owing to this, the results are in good agreement with that corresponding to stoicheiometric titration of manganese at both temperatures. The decrease in the slope at elevated temperature, although smaller than that found for mangesium, might also indicate a slow rate of reaction. The reproducibility of the titration is good.

TABLE III

OBSERVED VALUES FOR THE TITRATION OF MANGANESE

Value of α at the end-point	Temperature, °C	Slope	Intercept, millimolar	Standard deviation, millimolar	Number of determinations
0·8	20	1·0023 ± 0·0010	0·0013	0·0004	20
0·9	20	1·0043 ± 0·0012	0·0021	0·0005	20
0·8	60	1·000 ± 0·0012	0·0008	0·0004	10
0·9	60	1·001 ± 0·0016	0·0025	0·0006	10

DETERMINATION OF MANGANESE IN PRESENCE OF CALCIUM AND MAGNESIUM

The manganese, calcium and magnesium can be titrated together with EDTA, Eriochrome black T being used as indicator. Manganese can be removed from an aliquot by extraction with sodium diethyldithiocarbamate and carbon tetrachloride[17] and the sum of magnesium and calcium determined by the usual titration with EDTA. Manganese can then be calculated from the difference between the two titres.

PROCEDURE—

Stock solutions containing 2·2027 millimoles of calcium, 0·5485 millimoles of magnesium and from 0 to 1·486 millimoles of manganese per litre were prepared; the sum of these elements was determined on 10-ml portions by titration with EDTA.

Separate 25-ml portions of the stock solutions were adjusted to about pH 3 and diluted to 50 ml in calibrated flasks. The samples were transferred to separating funnels, about 50 mg of sodium diethyldithiocarbamate and a little solid hydroxylamine hydrochloride were added to each, and the funnels were shaken for a few minutes.[18] Separate 25-ml portions of carbon tetrachloride were then added, and the funnels were shaken for about 10 seconds; after a few minutes the carbon tetrachloride layers were run off. This procedure was repeated until the organic layers became colourless. Separate 20-ml portions of the aqueous layers were titrated for magnesium *plus* calcium. Manganese was then calculated from the difference between the two titres.

RESULTS—

Seven duplicate determinations of manganese were treated statistically by the method of least squares, leading to the regression equation—

$$C_Y = 0{\cdot}9889\ C_{Mn} + 0{\cdot}0015.$$

The residual standard deviation was 0·002, and the standard error of the slope was 0·0057. The results thus agree with those expected from a stoicheiometric titration.

REMARKS ON EXTRACTION PROCEDURE

Cheng, Melsted and Bray[18] proposed extraction with sodium diethyldithiocarbamate and *iso*amyl alcohol or chloroform for the removal of heavy metals. The choice of organic

solvent is important, as water may dissolve in the organic solvent and be removed, thus giving rise to an increase in concentration in the aqueous phase, and the organic solvent may dissolve in water, thus decreasing the concentration in the aqueous phase. Table IV shows observed residual volumes of aqueous phase based on the concentration change of a standardised magnesium solution after extraction with different amounts of *iso*amyl alcohol, together with theoretical values calculated from the solubility ratios.[19] The errors are expected to be less if chloroform is used instead of *iso*amyl alcohol, and still less if carbon tetrachloride is used. With the latter, no error could be observed in my experiments.

TABLE IV

VOLUME OF AQUEOUS PHASE AFTER ONE EXTRACTION WITH *iso*AMYL ALCOHOL

Ratio by volume of *iso*amyl alcohol to water	Volume of aqueous phase—	
	calculated, ml	found, ml
20 to 100	102·0	102·2
40 to 100	100·2	100·8
100 to 100	95·4	95·4

I express my appreciation to D. L. Lydersen for valuable discussions and K. Rutherford for linguistic help in preparing this paper.

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The Use of Ion-exchange Enrichment in the Determination of Trace Elements in Sea Water

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Some extremely rare elements have been determined spectrographically in sea water enriched by an ion-exchange procedure. This procedure depends on the fact that none of the major constituents of sea water can form anionic chloro complexes in the presence of small amounts of hydrochloric acid, whereas many of the trace elements, such as bismuth and thallium, form strong complexes in 0·1 *N* hydrochloric acid.

The sea water (0·25 ton) was treated with sufficient redistilled hydrochloric acid to make the solution 0·1 *N* and was allowed to flow very slowly through a small column of a strongly basic anion-exchange resin (Amberlite IR-400). After this sorption stage, the column was washed, and some of the trace elements were eluted. The resin itself was ignited, and the ash and effluent were analysed spectrochemically.

By this technique, enrichment factors of up to 2×10^7 were achieved. Risk of contamination was small, as only one reagent was used throughout and this was easily purifiable. A further advantage of the method was that the amount of hydrochloric acid added to form complexes with the trace elements was much less than would have been necessary if a non-saline water had been used. This is because the concentration of chloride in sea water was high enough to assist substantially in the formation of the complexes.

Results for the abundance of bismuth, gold and cadmium in sea water are reported.

In the presence of hydrochloric acid, certain elements can form strongly anionic chloro complexes; these complexes were extensively investigated by Kraus and Nelson,[1] who measured the volume distribution coefficients (D_v) of nearly all the elements of the periodic table when their solutions in different concentrations of hydrochloric acid were brought into equilibrium with the strongly basic anion-exchange resin Dowex 1. A striking characteristic of their results is the fact that none of the common elements in the earth's crust can form a strong chloro complex in solutions 2 *N* in hydrochloric acid, whereas many of the trace elements form very strong complexes. The elements forming strong complexes in 2 *N* hydrochloric acid are listed below in order of decreasing magnitude of D_v. Unless otherwise stated, the values refer to the highest oxidation state of the metal.

Au ($D_v = 10^6$), Tl, Hg, Bi, Sb^{3+}, Sn, Cd, Zn, Re, Ag ($D_v = 100$).

In addition, the platinum metals all form strong complexes. Iron forms a very weak complex ($D_v = 10$) and can therefore be easily separated from other more strongly adsorbed elements.

Techniques for the anion-exchange enrichment of trace elements in silicate rocks have been developed, in which the chloro complexes of the trace elements are adsorbed on an anion-exchange column and separated from the major constituents of the rock.[2,3,4,5] In considering the application of these techniques to the determination of trace elements in sea water, it was obvious that large enrichment factors would have to be attained for the determination of extremely rare elements, such as cadmium, bismuth or gold. I was, in fact, able to achieve enrichment factors of up to 2×10^7, owing to three important factors. First, since the "sample" was already in solution no reagents had to be used to effect solution. Secondly, the values of the distribution coefficients for the elements to be studied (gold, bismuth, cadmium and possibly thallium) were so great that only a small column was necessary to effect adsorption from a large volume of sea water. Thirdly, the most important factor was that, since sea water is already 0·5 *M* in chloride ion, the formation of chloro complexes was likely even without the addition of hydrochloric acid. The acid was added, however, to minimise the danger of hydrolysis or reduction of the trace constituents, but the amount used was much less than would have been necessary for a non-saline water.

In order to facilitate the handling of extremely large amounts of sea water, the operations were carried out at premises on the sea-front.

Description of apparatus

The apparatus used is shown in Fig. 1. The sample was contained in a 10-gallon carboy and was fed into the first of two sedimentation bottles by means of an arrangement whereby a constant level of water was maintained in the bottle. The first sedimentation bottle was connected to a second by a siphon tube, and the second bottle fed the ion-exchange column via a second siphon tube. The siphon tubes were also connected to small inverted bottles to obviate "breaking" of the siphon by bubbles of gas in the sea water (only one of these inverted bottles is shown in Fig. 1). Sedimentation was an efficient means of removing solids suspended in the sea water. As an added precaution, a cotton-wool plug was placed in the column to effect further filtration.

The ion-exchange column was of small dimensions (0·5 sq. cm × 13·2 cm), and it was calculated that such a column would quantitatively absorb from 250 litres of sea water those elements having distribution coefficients greater than 38,000, *i.e.*, thallium, bismuth, gold, etc.

A Hilger large-quartz spectrograph was used for the final determinations.

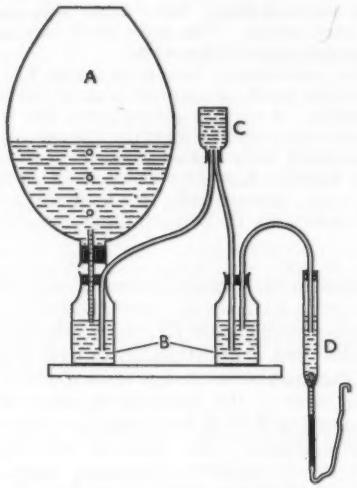

Fig. 1. Diagram of apparatus used for determining trace elements in sea water: A, 10-gallon carboy; B, sedimentation bottles; C, siphon bottle; D, ion-exchange column

Preliminary experiments

Although Kraus and his co-workers obtained comprehensive results for the distribution coefficients of zinc, bismuth, gold, thallium and cadmium, their work was carried out with pure solutions in hydrochloric acid, and it was to be expected that the chloride content of sea water would radically affect the values of the coefficients for a given concentration of acid. A knowledge of the precise values of D_V for zinc and cadmium under these conditions was necessary before these elements could be determined quantitatively in sea water. The values of the distribution coefficients for gold, thallium and bismuth were so great that the chloride content of sea water was not likely to decrease them below the critical value (38,000).

The precise values of D_V for cadmium and zinc in acidified sea water were determined as follows. A 1-g portion of dried ion-exchange resin (Amberlite IR-400) was placed in each of four 100-ml conical flasks, and to the resin in two of the flasks were added 50 ml of 0·001 *N* hydrochloric acid and about 500 μg of cadmium or zinc. The remaining two portions of resin were similarly treated, except that the acid was prepared in sea water instead of distilled water. The flasks were agitated for 24 hours, and aliquots of their contents were analysed

absorptiometrically with dithizone.[6] The acidities of the solutions were then adjusted to 0·01 *N* by adding hydrochloric acid and sufficient distilled or sea water to restore the volume to 50 ml. This procedure was repeated with 0·1, 1, 2 and 3 *N* hydrochloric acid.

From the results so obtained were plotted curves showing the distribution coefficients as a function of acidity. The curves for zinc and cadmium are shown in Fig. 2, the results of Kraus and his co-workers being also included. These curves show that the high concentration of chloride in sea water results in greater values for the distribution coefficients at low concentrations of hydrochloric acid than would otherwise be obtained.

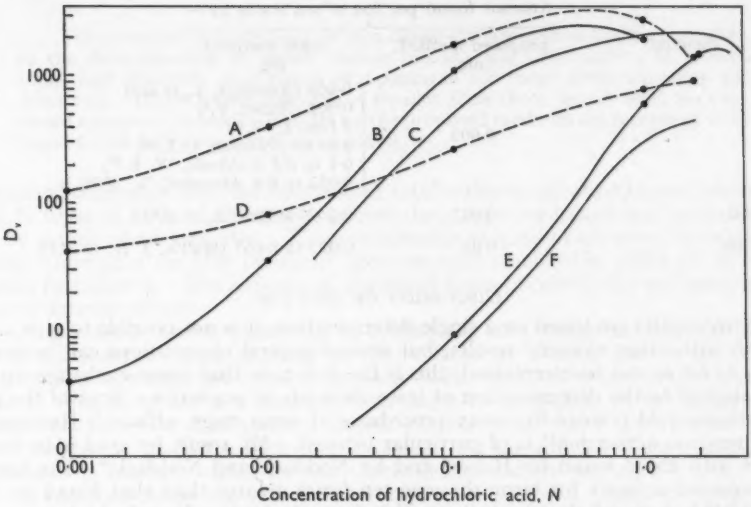

Fig. 2. Distribution coefficients for cadmium and zinc in hydrochloric acid and in sea water acidified with hydrochloric acid: curve A, cadmium in sea water; curves B and C, cadmium in hydrochloric acid; curve D, zinc in sea water; curves E and F, zinc in hydrochloric acid. (Curves C and F plotted from results found by Kraus and Nelson[1])

Determination of gold, bismuth and cadmium in sea water

This experiment was carried out with 250 litres of sea water, to which was added sufficient redistilled analytical-reagent grade hydrochloric acid to adjust the acidity to 0·1 *N*. The acid could have been purified by an ion-exchange technique, but as this involved prior purification of the resin by elution, distillation was preferred. Tests on the purified acid showed that cadmium and gold were absent, but small traces of bismuth and zinc were present. However, the amount of bismuth present was later found to be only 10 per cent. of the amount found in the sea water.

After acidification, sufficient bromine water was added to the sea water to adjust the concentration of this element to about 10 p.p.m. This was to ensure that any thallium was present in the thallic state (thallous ion is not adsorbed), to prevent reduction of the metals by the resin and to attempt to lessen the danger of adsorption of the gold on the walls of the containing vessel.

The sea water was then passed through the apparatus shown in Fig. 1; the flow rate was 120 ml per hour, and about 100 days were required for the sorption stage. After adsorption, the column was washed with 2 *N* hydrochloric acid and then eluted with 1 litre of 0·25 *N* nitric acid. The effluent was evaporated to dryness and collected in the sodium chloride matrix. The resin itself was ignited, and the ash was collected in a similar matrix. The two samples were analysed spectrographically, together with the blank.

Distinct lines were detected for gold (at 2676 A) in the resin fraction and for bismuth, cadmium and zinc (at 3067, 3261 and 3345 A, respectively) in the effluent fraction. Thallium appeared to be absent, and the line for zinc was somewhat diffuse, which precluded its accurate measurement. The antimony line at 3267 A was used as an internal standard,[7] and working

curves were prepared for the determination of the cadmium, gold and bismuth. Results for the abundance of bismuth and gold in sea water were obtained directly from these curves. These results, together with those of other workers for comparison, are shown in Table I. Since the column was not long enough quantitatively to retain all the cadmium, a figure greater than 0·02 mg per ton is reported for this element.

TABLE I

ABUNDANCE OF GOLD, BISMUTH AND CADMIUM IN SEA WATER

Element	Amount found per ton of sea water by— proposed method, mg	other methods, mg
Gold	0·009	0·008 (Noddack, I., *et al.*[8]) 0·004 (Haber, F.[9]) 2 (Stark, W.[10]) 0 to 46 (Putnam, C. L.[11]) 0·1 to 0·2 (Caldwell, W. E.[12]) 0·015 to 0·4 (Hummel, R. W.[13])
Bismuth	0·017	0·2 (Noddack, I., *et al.*[8])
Cadmium	>0·02	0·032 to 0·057 (Mullin, J. B., *et al.*[14])

DISCUSSION OF RESULTS

Since my results are based on a single determination, it is not possible to give a detailed comparison with other workers' results, but several general observations can be made.

First, as far as can be ascertained, this is the first time that anion-exchange enrichment has been applied to the determination of trace elements in sea water. Most of the methods for determining gold involve fire-assay procedures at some stage, although Hummel's work involving neutron activation[13] is of particular interest. My result for gold is in reasonable agreement with those found by Haber[9] and by Noddack and Noddack.[8] The last-named workers reported a figure for bismuth some ten times greater than that found by me, but Goldschmidt[15] has stated that he believes this figure to be inordinately high in proportion to the amount of bismuth in rocks and the solubility of its compounds as compared with those of other elements.

It is hoped soon to extend the investigations described in this paper to other elements and to obtain further results for bismuth and cadmium, since knowledge of the abundance of these elements in sea water is at present incomplete.

I thank Mr. J. J. du Plessis, Division of Fisheries, for permission to use their laboratory facilities at the sea-front, Sea Point, Cape Town. I also thank Professor L. H. Ahrens, Department of Chemistry, University of Cape Town, for valuable encouragement and advice.

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Closed-circulation Systems for Determining Water, Carbon Dioxide and Total Carbon in Silicate Rocks and Minerals

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The principle of re-cycling air in a closed-circuit system has been applied to the determination of water, carbon dioxide and total carbon in silicate rocks and minerals, and pieces of apparatus for these determinations are described. Blank values are always smaller than those found when conventional apparatus is used. Results by the proposed methods are compared with those found by conventional procedures.

In the method commonly used for determining total water in silicate rocks and minerals,[1,2,3,4] as much as 10 to 15 litres of air are drawn over the sample contained in a heated silica tube, usually by means of an aspirator. This considerable volume of air must be carefully dried by passage through a suitable desiccant, such as calcium chloride, silica gel or anhydrous magnesium perchlorate. The last-named absorbent tends to cake badly and must be replaced after a few determinations.

By using a small electric pump and re-cycling the air through the apparatus, the large amount of desiccant normally required can be replaced by a much smaller amount of magnesium perchlorate, which can be used for many determinations before being replaced. The principle of circulating air in a closed system can also be applied to the determination of "moisture" (water evolved at 105° C), carbon dioxide, total water (by a fusion procedure) and total carbon (by oxidation with chromic acid). As the amount of air circulating within the apparatus is limited, values for the blank determinations are always small compared with those obtained when the conventional apparatus is used. Closed-circulation systems are therefore preferable, especially for determinations on a reduced or semi-micro scale.

Determination of water evolved at 105° C

Method—

For this determination, the apparatus shown in Fig. 1 has been devised. It consists of a closed-circulation system comprising a small electric pump,† a heating chamber maintained at 104° to 106° C by means of boiling *iso*butyl alcohol, two absorption tubes and a bubbler containing orthophosphoric acid to indicate the rate of flow of air through the apparatus. Before inserting the sample, tube A is replaced by a short piece of glass tubing, and the air in the system is then dried by operating the pump for about 30 minutes. Tube A is weighed and replaced, the sample is inserted, and the circulation of air is continued until all water evolved from the sample has passed into the absorption tube. In practice, the apparatus is kept running for about 2 hours, as this period has been found sufficient to drive over all the water, including any that may condense on the cooler parts of the heating chamber. The blank value, *i.e.*, the increase in weight of absorption tube A when the determination is carried out without the insertion of a sample, is less than 0·1 mg.

Tube A, which is used in place of the more conventional U-tube, contains a paper spiral that prevents clogging of the magnesium perchlorate when large amounts of water are absorbed. The open end of the tube, fitted with a B7 ground-glass joint, is closed with a cap during weighing. When fully packed, the tube and cap together weigh 20 to 25 g.

Discussion of results—

The conventional method of determining water evolved at 105° C is to dry the sample to constant weight by heating in an electric oven or in a toluene bath.[1,2,3] This procedure is

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† Several small-capacity pumps have been used; the most satisfactory was obtained from Boughton Pumps Ltd., Effingham, Surrey (model F43).

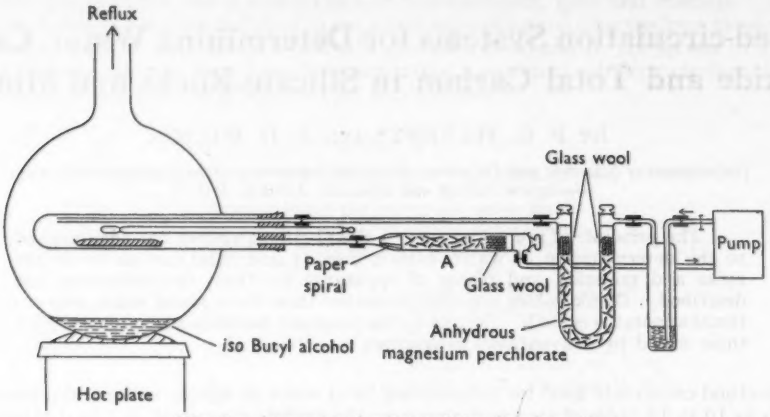

Fig. 1. Apparatus for determining "moisture"

simple and gives results in agreement with those found by the proposed method (see Table I); its use is therefore generally to be preferred. The loss in weight measured by such a method, however, will be affected by the relative humidity of the atmosphere at the time. Further, certain rocks, *e.g.*, those containing chlorite, are very hygroscopic and are difficult to weigh accurately after drying. For such rocks and for those in which ferrous iron readily undergoes oxidation, the proposed procedure is to be preferred.

TABLE I

"MOISTURE" CONTENTS OF SOME SILICATE ROCKS

Samples were taken from the collection of analysed rocks, Geological Survey of Great Britain; the numbers are laboratory serial numbers

	Rock material	Loss in weight after heating at 105° C,* %	Water found by proposed method, %
1007.	Granite, Miterdale, Cumberland	0·12	0·13
1771.	Granite, Burn of Roerwater, Shetland	0·20	0·21
1042.	Tonalite, Strontian, Argyllshire	0·75	0·74
1004.	Augite-andesite, Carrick Hills, Maybole, Ayrshire	1·03	1·00
1527.	Olivine-basalt, Arngask, Perthshire	1·62	1·65
1478.	Albite-diabase, borehole near Caunton, Nottinghamshire	1·36	1·36
1744.	Altered basalt (now largely chlorite), Fallgate borehole, Derbyshire	3·77	3·69
1480.	Picrite, Long Clawson No. 1 borehole, Leicestershire	5·19	5·17

* These results are new determinations and not previously published figures, as some powdered rocks have variable moisture contents, *e.g.*, those containing chlorite.

DETERMINATION OF TOTAL WATER

METHOD—

The apparatus used for determining total water is shown in Fig. 2 and is based on the conventional apparatus. It consists of a silica tube containing the sample in a porcelain boat, which is heated to above 1000° C in a gas-fired furnace. A current of air from a small electric pump is passed into an absorption tube containing magnesium perchlorate and soda asbestos, over the sample and then through a weighed absorption tube (of the type described above) containing magnesium perchlorate. The circulation is completed through a bubbler containing orthophosphoric acid, which indicates the rate of flow of air through the apparatus, and finally back to the pump. The end of the silica tube is separately heated to between 300° and 400° C and is packed with basic lead chromate, which retains oxides of sulphur[5]; copper wire and silver pumice have also been used[6] for this purpose. The wide end of the

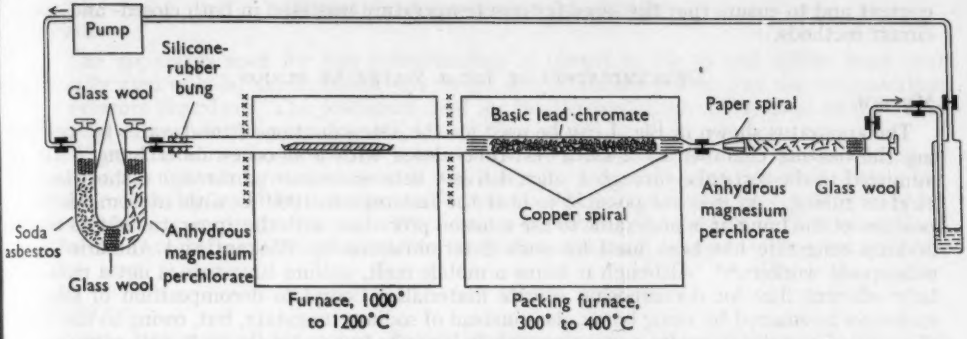

Fig. 2. Apparatus for determining total water

silica tube is closed with a silicone-rubber bung, the substitution of which for the rubber bung previously used in this apparatus made it possible to increase the temperature of the furnace to over 1000° C, as silicone rubber is more resistant to heat than is ordinary rubber.

The air within the apparatus is first dried by operating the pump for 1 hour, during which time the packing heater reaches its operating temperature. The weighed sample is then inserted, and the determination is completed in the usual way.[3,4] The blank value, *i.e.*, the gain in weight of the absorption tube when the sample is omitted, is less than 0·1 mg per determination. For the conventional apparatus described by Groves,[4] the blank value is 1·7 to 2·0 mg, although this can be reduced to about 0·5 mg by closing the silica tube with a silicone-rubber bung.

DISCUSSION OF RESULTS—

Results for the water contents of some silicate rocks by the proposed method are shown in Table II, together with the corresponding results by the conventional method[4] and by the fusion method described on p. 752.

TABLE II

TOTAL-WATER CONTENTS OF SOME SILICATE ROCKS

Samples were taken from the collection of analysed rocks (see Table I)

	Rock material	Water content found by— conventional method,[4] %	proposed method, %	fusion method, %
1771.	Granite, Burn of Roerwater, Shetland	0·46	0·46	0·46
1733.	Tonalite, Clounlaid, Morvern, Argyllshire	1·35	1·34	1·40
1628.	Lamprophyre, Salen, Lock Sunart, Argyllshire	2·43	2·47	2·50
1697.	Rhyolite, 1700 yards N.W. by E. from Hope Bowdler Church, Church Stretton, Shropshire	3·62	3·62	3·62
1448.	Picroteschenite, Lugar Sill, Lugar, Ayrshire	4·22	4·22	4·22
1698.	Andesite, Middle Hill, near Church Stretton, Shropshire	4·58	4·58	4·60
1612.	Porphyritic obsidian, Sandy Braes borehole No. 3, Tardree Mountain, Co. Antrim	12·82	12·80	12·90
	Staurolite, St. Gotthard	1·52	1·51	1·62
	Topaz, unknown locality	—	0·73*	0·86
	Epidote, unknown locality	—	2·12	2·12

* Sample heated for 15 hours.

To render results by the different methods strictly comparable, those results obtained by the conventional method represent determinations carried out at the same time as determinations by the other methods so as to eliminate changes due to fluctuations in moisture

content and to ensure that the same furnace temperature was used in both closed- and open-circuit methods.

Determination of total water by fusion

Method—

The apparatus shown in Fig. 1 can be used for the determination of total water by replacing the heating chamber by a silica test-tube closed with a silicone-rubber bung. Air is admitted to the test-tube through a silica delivery tube and removed through a short length of glass tubing. As it is not possible to heat the test-tube to 1000° C without some decomposition of the bung, it is preferable to use a fusion procedure with the apparatus in this form. Sodium tungstate has been used for such determinations by Wiskont and Alimarin[7] and subsequent workers.[4,8] Although it forms a mobile melt, sodium tungstate is not a particularly efficient flux for decomposing silicate materials. Complete decomposition of silicate rocks can be ensured by using borax glass instead of sodium tungstate, but, owing to the high viscosity of borosilicate melts, water vapour is liable to be trapped in the melt, with consequent low recoveries of total water.

By combining sodium tungstate and borax glass in equal proportions, complete fusion is readily attained; the melt is fluid and does not retain entrapped bubbles of water vapour, and decomposition of the sample is complete, as is shown by the transparent melt. For decomposing a 0·5-g sample of silicate rock, 2 g each of borax glass and anhydrous sodium tungstate are needed. The fluxes and the sample are mixed in a platinum boat, which is then inserted into the silica tube and heated to about 800° C. The decomposition of even refractory silicates, such as staurolite, is complete in about 30 minutes. In practice, the air circulation is continued for 1 hour to ensure collection of all the water vapour evolved.

Discussion of results—

Results found by fusion with sodium tungstate and borax, as described above, for a number of rock and mineral samples are shown in Table II. For most of the rocks examined, results by the fusion procedure are in good agreement with those found by other methods. When discrepancies exist, results by the fusion procedure are higher, illustrating the well known difficulty of removing all the water from refractory silicates by ignition.

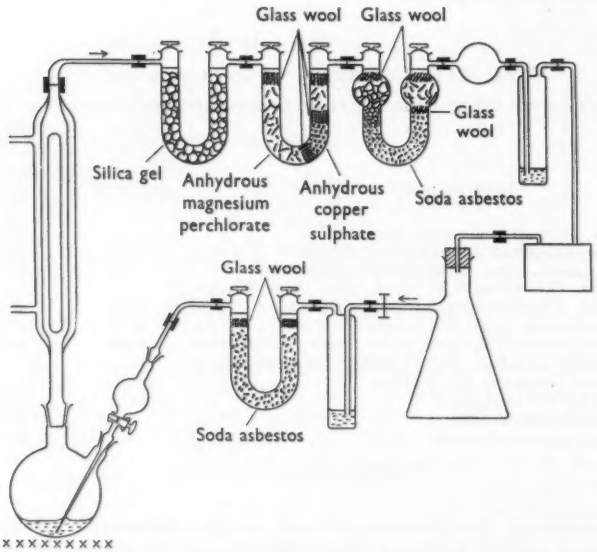

Fig. 3. Apparatus for determining carbon dioxide

Determination of carbon dioxide

Method—

The apparatus used for this determination is shown in Fig. 3 and differs from that generally used only by the inclusion of a closed air-circulation system and the incorporation of a pressure chamber. The procedure used for the determination is the same as that used with the conventional apparatus.

When diluted orthophosphoric acid (1 + 3, v/v) was used for the decomposition of silicate rocks containing carbon dioxide, the average blank value was less than 0·1 mg per determination; hydrochloric acid, generally used for this determination, gave a reagent blank value of 0·8 mg per determination. These values should be compared with the reagent blank value of 1·5 to 2·0 mg per determination for the conventional apparatus when hydrochloric acid is used.

Results—

Some results found for carbon dioxide by using the closed-circulation system are shown in Table III, together with results found by the method described by Harwood,[9] in which diluted orthophosphoric acid was used.

Table III

Carbon dioxide contents of some silicate rocks

Samples were taken from the collection of analysed rocks (see Table I)

	Rock material	Carbon dioxide found by— method described by Harwood,[9] %	Carbon dioxide found by— proposed method, %
1064.	Granite, Voe, Delting, Shetland	0·10	0·11
1447.	Nepheline-syenite, borehole No. 16, Cronberry, Lugar, Ayrshire	0·26	0·26
1009.	Quartz-diorite, Close Quarry, Embleton, Cockermouth, Cumberland	0·57	0·58
1628.	Lamprophyre, Salen, Lock Sunart, Argyllshire	0·81	0·86
1071.	Camptonite, Allt Feith Mhic Artair, Kingairlock, Argyllshire	1·26	1·26
1617.	Black Ven marl, Bridport, Dorsetshire	7·62	7·65
1327.	Altered quartz-dolerite, Whin Sill, Settlingstones Mines, Newbrough, Northumberland	14·75	14·75

Determination of total carbon

Method—

Dixon's orthophosphoric - chromic acid method[10] for determining total carbon can be modified by incorporating a closed-circulation system. With the addition of a second flask, as shown in Fig. 4, to retain water distilling from the acid mixture, the closed-circulation

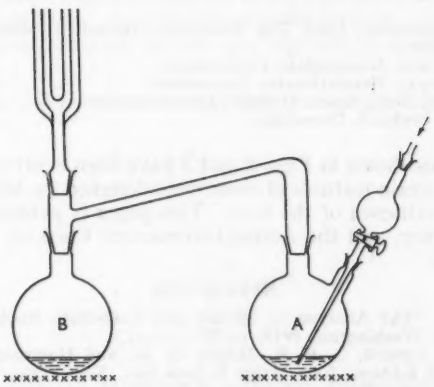

Fig. 4. Apparatus for determining carbon

apparatus used for determining carbon dioxide (see Fig. 3) can be used for the determination of both carbon dioxide and total non-carbonate carbon.

A 2-g sample of rock material is weighed into flask A (see Fig. 4), and 5 ml of water are placed in flask B. This water is heated under gentle reflux to prevent the accumulation of carbon dioxide in this part of the apparatus, and the determination of carbon dioxide is completed in the usual way, 30 ml of diluted orthophosphoric acid (1 + 3, v/v) being used. When this stage of the determination is complete, the weighed absorption tube is replaced, 30 ml of orthophosphoric - chromic acid mixture are added, and the determination of non-carbonate carbon is completed as described by Dixon. Water from the diluted orthophosphoric acid used in the first part of the determination is collected in flask B and kept boiling throughout the remainder of the determination. Towards the end of the distillation from flask A, the colour of the contents changes from yellow-brown to green. The distillation should be discontinued before this change is complete, as further heating at this stage results in an excessive reagent blank value. With the closed-circulation system, the reagent blank value was 0·7 mg per determination, compared with 2 to 3 mg for Dixon's procedure.

Low recoveries of total carbon were noted for some silicate rocks containing considerable amounts of graphite. Dixon used two reaction flasks to ensure complete oxidation, but the closed-circulation system should not require a second flask, and we attribute our losses to the tendency of graphite particles to adhere to the upper parts of the flask and so to escape oxidation. For such samples, it is better to determine the carbonate content of the material separately and, in the determination of total carbon at the conclusion of the oxidation, to remove flask A, rinse its walls with water, add a further 30 ml of orthophosphoric - chromic acid mixture, and repeat the oxidation.

Discussion of results—

Some results of determinations of non-carbonate carbon in silicate rocks are shown in Table IV, together with results by Dixon's procedure. Agreement between the results by the two methods is satisfactory, although for some rocks slightly higher results were obtained by the proposed procedure. This is possibly caused by oxidation of some of the graphite to carbon monoxide, which, in Dixon's procedure, is not collected. In the closed-circulation procedure, passage through the boiling orthophosphoric - chromic acid mixture would complete the oxidation of any carbon monoxide formed during the initial oxidation.

Table IV

Non-carbonate carbon contents of some silicate rocks

Samples were taken from the collection of analysed rocks (see Table I)

	Rock material	Carbon found by— Dixon's method,[10] %	Carbon found by— proposed method, %
1433.	Metamorphic limestone, Lock Tay limestone, Dalradian Series, Cowall, Argyllshire	0·17	0·19
1036.	Slate, Skiddaw Slate, Mungrisdale, Cumberland	0·39	0·39
1034.	Slate, Skiddaw Slate, Bassenthwaite, Cumberland	0·39	0·39
1017.	Sillimanite-gneiss, Beinn Gaire, Moidart, Inverness-shire	1·10	1·11
1617.	Black Ven marl, Bridport, Dorsetshire	1·83	1·84

The sets of apparatus shown in Figs. 2 and 3 have been evolved over a number of years in this laboratory, and certain features of them were designed by Mr. C. O. Harvey, to whom we are also grateful for criticism of the text. This paper is published by permission of the Director, Geological Survey, and the Acting Government Chemist, Department of Scientific and Industrial Research.

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Recommended Methods of Assay of Crude Drugs

PREPARED BY THE JOINT COMMITTEE OF THE PHARMACEUTICAL SOCIETY AND THE SOCIETY FOR ANALYTICAL CHEMISTRY ON METHODS OF ASSAY OF CRUDE DRUGS

Assay of Rauwolfia

In 1956 the Pharmaceutical Society of Great Britain and the Society for Analytical Chemistry formed a Joint Committee to investigate methods of assay of crude drugs. Early in 1957 a working Panel was appointed to examine methods of assay of rauwolfia; the Panel consisted of Mr. C. A. Johnson (Chairman), Mr. T. Davies, Mr. F. G. Farrell, Mr. J. J. Lewis and Mr. A. W. Peacock, with Miss A. M. Parry as Secretary. Mr. Farrell resigned from the Panel at the end of 1958 and was succeeded by Miss B. Gartside.

The Panel's terms of reference were—

"To investigate methods of assay for rauwolfia and its preparations with particular regard to the content of reserpine and related alkaloids."

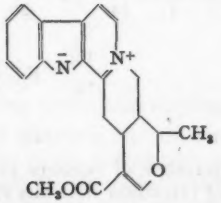
This report of the Panel describes a recommended method of assay for rauwolfia root and extract and discusses its limitations. It also describes certain other approaches that have been made to the determination and gives the reasons why these methods were abandoned.

Report

Alkaloids of rauwolfia—

Rauwolfia is principally used in Great Britain for the extraction of reserpine, and, although a large number of species has been reported in the literature, the main source materials are *R. serpentina* and *R. vomitoria*. Species of rauwolfia contain a considerable number of alkaloids that are mainly indole derivatives and may logically be divided into four groups, as shown below.

(*i*) The strongly basic yellow-coloured anhydronium compounds, typical of which are serpentine (**I**),

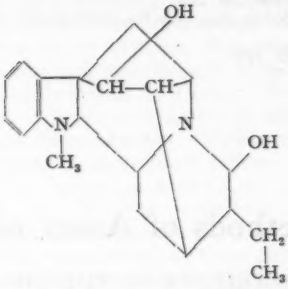


(**I**)

alstonine (a stereo-isomer of **I**) and serpentinine. The pK_a values of these bases are from 10·4 to 11·0. The pharmacological action of this group of alkaloids has been variously reported

by different workers, and it seems generally agreed that they do not have the characteristic tranquillising, sedative or hypnotic action of the whole root.

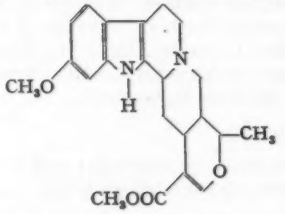
(*ii*) The moderately basic tertiary indoline compounds, of which the most abundant is ajmaline (**II**).



(**II**)

Others are *iso*ajmaline, rauwolfinine, semperflorine and tetraphyllicine. The pK_a values of bases of this type are from 8·15 to 8·3. Although somewhat different from one another in their mode of pharmacological action, they are all reported as lacking the tranquillising and sedative action of the crude drug itself.

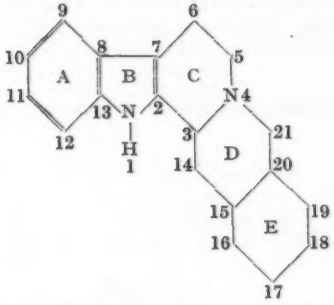
(*iii*) The weakly basic indole alkaloids with a heterocyclic ring E (for ring numbering and lettering, see the next group). Typical of these is reserpinine (**III**).



(**III**)

Ajmalicine (γ-yohimbine) is 11-desmethoxyreserpinine. Others are aricine (CH_3O— group in the 10- instead of 11-position) and reserpiline (CH_3O— groups in the 10- and 11-positions). The pK_a values of this group of alkaloids are between 6·0 and 7·5. Few pharmacological data exist for this group of alkaloids, which appear to make little or no contribution to the tranquillising and sedative action of rauwolfia.

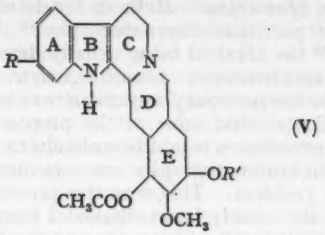
(*iv*) The weakly basic alkaloids with a carbocyclic ring E. The fundamental ring skeleton of these alkaloids is—



(**IV**)

Members of this group are a number of isomers of yohimbine and also sarpagine or raupine, the only phenolic alkaloid of rauwolfia that has so far been isolated. Again, there is little pharmacological interest in this group of alkaloids.

A subdivision of the group, and all-important pharmacologically and commercially, consists of alkaloids having the general structure (**V**) and esterified at position 18.



(V)

These consist of reserpine ($R = OCH_3$; $R' = -OC-$ with OCH_3 at 3, 4, 5), rescinnamine ($R = OCH_3$; $R' = -OC-CH=CH-$ with OCH_3 at 3, 4, 5) and deserpidine* ($R = H$; $R' = -OC-$ with OCH_3 at 4, 5). As for the other weakly basic indole alkaloids, the pK_a values are in the region of 6 to 7.

PUBLISHED METHODS OF ASSAY OF RAUWOLFIA—

Many methods have been suggested for the assay of rauwolfia, and it would be impracticable to refer to them all. The main approaches, with representative examples, are described below.

1. *Determination of total alkaloids*—This is the method given in the British Pharmaceutical Codex, 1959,[1] although it seems probable that the weakly basic anti-hypertensive alkaloids would not be included in the "total" figure.[2] Since reserpine forms only a small proportion of the total alkaloids present and since the ratio of reserpine to other alkaloids may vary greatly from sample to sample, this general method was not considered by the Panel.

2. *Separation and determination of the weakly basic alkaloids as a group*—The separation methods described in the literature are based on (*a*) separator extraction methods[3,4] or (*b*) liquid - liquid partition chromatography.[5]

When separated, the anti-hypertensive alkaloids, which in *R. serpentina* and *R. vomitoria* are reserpine and rescinnamine, have been determined by one of the procedures listed below.

(*i*) Fluorimetrically, after heating the alkaloids with sulphuric acid,[6] mixtures of acids[7] or hydrogen peroxide.[8]

(*ii*) By dye-extraction.[9]

(*iii*) By reaction with vanillin.[10]

(*iv*) By reaction with sodium nitrite and sulphuric acid.[4,11,12]

Determination of the combined reserpine and rescinnamine content of rauwolfia should, if satisfactory, provide a useful index to the therapeutic and commercial value of the sample. After a consideration of these methods, the Panel decided that the separator extraction method and subsequent colorimetric determination with sodium nitrite, offered most promise and should be tested.

* Also commonly known as canescine or recanescine.

3. *Complete separation of reserpine*—Methods for determining reserpine alone have been based on (*a*) liquid - liquid partition chromatography,[13] (*b*) counter-current extraction[14,15] and (*c*) paper ionophoresis,[16] the alkaloid being usually determined in the separated fractions by ultra-violet absorption spectroscopy. Counter-current methods could not be subjected to collaborative test because the necessary apparatus was not available. Published work on approaches (*a*) and (*c*) indicates that most of the proposed methods are tedious, and the Panel considered that such procedures would be unlikely to find favour as routine methods of assay. One use of partition chromatography was examined, since it seemed a simple and ingenious approach to the problem. This was the procedure described by Carol and his co-workers,[5] who separated the weakly basic alkaloidal fraction on a column, hydrolysed the alkamine esters, extracted the trimethoxybenzoic and trimethoxycinnamic acids derived from the reserpine and rescinnamine, respectively, and subsequently determined these two acids, and hence the alkaloids, by using a spectrophotometric method and employing a two-point correction procedure.

The collaborative work of the Panel was therefore concerned with two possible methods—

A. Separation of the weakly basic alkaloids and then hydrolysis and spectrophotometric determination of the resulting acids.

B. Separation of the weakly basic alkaloids and then colorimetric determination with nitrite.

Experimental

Examination of method based on hydrolysis and spectrophotometric determination of resulting acids—

After variable results had been obtained by the Panel when the published method[5] was applied to rauwolfia, an examination was made of the ultra-violet absorption characteristics of trimethoxybenzoic and trimethoxycinnamic acids. This led to the discovery that solutions of the latter acid and also of rescinnamine undergo rapid and considerable change in ultra-violet absorption characteristics when exposed to daylight. Full details of the work of the Panel in this respect have already been published.[17] A stronger light source causes more complete decomposition of both rescinnamine and reserpine, as shown by Ljungberg.[18] It was concluded that, without modification, this method of assay proposed by Carol and others was not reliable.

In an extension of this investigation, attempts were made to assay solutions containing a mixture of trimethoxybenzoic and trimethoxycinnamic acids by irradiating the solutions with ultra-violet light until the light-absorption characteristics became constant. Results were calculated by reference to extinction values obtained for similarly irradiated standard solutions of the individual acids. All members of the Panel obtained good recoveries of the two acids from mixed solutions, but there were differences in the reported extinction values of the standards, probably because of variation in irradiation conditions. When attempts were made to apply this principle to the determination of rauwolfia itself, results were unsatisfactory, and it was thought that a considerable amount of work would be required before a reliable procedure could be recommended. Such work would be more suitable for individual investigation than collaborative trial, and it was therefore decided to abandon this approach.

Examination of method based on extraction of weakly basic alkaloids and then colorimetric determination by the sodium nitrite procedure—

The colour is developed by the addition of dilute sulphuric acid and sodium nitrite solution to an ethanolic solution of reserpine. After being set aside for some time, the solution develops a greenish yellow colour, which has a maximum light absorption at about 390 mμ. This method was first described and examined by Szalkowski and Mader[11]; later, Haycock and Mader[12] showed that the 11-methoxy group and the A, B and C ring skeleton of reserpine (see formula **V**) are necessary for the formation of the coloured complex. It follows that the reaction has a considerable degree of specificity, and it has been shown that many other indole alkaloids, such as yohimbine and strychnine, do not respond. Among the weakly basic alkaloids of rauwolfia, both reserpine and rescinnamine give the reaction, but deserpidine (11-desmethoxyreserpine) does not. The last-named alkaloid, which has anti-hypertensive activity, has not been reported in either *R. serpentina* or *R. vomitoria*, but both reserpine and rescinnamine are present, and their pharmacological activities have been reported by various

workers to be similar.[19,20,21] The first stage of the Panel's work, therefore, was to carry out a collaborative test on the nitrite procedure as applied to pure reserpine and to compare the results with those given by rescinnamine.

At first, a method based upon that of Szalkowski and Mader was applied. A solution of the alkaloid in ethanol was treated with dilute sulphuric acid and sodium nitrite solution and was set aside for 1 hour. The excess of nitrite was then destroyed by addition of sulphamic acid, and, after adjustment to a definite volume, the extinction value of the solution was measured at 390 mμ. In practice, it was found that 100- to 300-μg amounts of reserpine in a volume of 20 ml gave suitable extinction values.

Work by members of the Panel showed, however, that 1 hour at laboratory temperature was not sufficient for development of the colour, and a time of 3 hours was agreed upon. Good agreement was obtained within each laboratory for the $E^{1\%}_{1cm}$ values of the final coloured solutions, but inter-laboratory results varied between 360 and 410. Similar results were obtained when a standard solution of rescinnamine was treated by the same procedure. It was thought that the differences between laboratories might be due to variations in instruments, and a comparison was made by circulating an alkaline solution of potassium chromate and three high-grade, N.P.L.-checked filters—Chance OV2(A), OV2(B) and OX1. Results obtained on the chromate solution were variable, but those on the filters showed agreement within 1 per cent. At this stage it was agreed that each laboratory should prepare its own calibration graph and calculate results by reference to it.

Samples of reserpine and rescinnamine were next subjected to the proposed extraction and separation procedure for rauwolfia root, based on that of Banes and his co-workers.[3] This consists in extraction in a Soxhlet apparatus with ethanol for 4 hours, dilution of an aliquot with 0·5 *N* sulphuric acid and then extraction of the aqueous ethanol with trichloroethane, which is discarded. The reserpine and rescinnamine are then separated from other alkaloids by extracting the acid solution with chloroform. A suitable aliquot of the chloroform solution is evaporated and used for the colour-development stage. Recoveries of reserpine were complete, but those for rescinnamine were about 2 to 3 per cent. low. The Panel agreed that recoveries of this order were satisfactory.

When the method was applied to samples of rauwolfia root, variations of up to $\pm$15 per cent. were obtained, and it was therefore decided to examine the conditions of extraction and colour development in greater detail. The points investigated were—

(*i*) *Time of extraction in Soxhlet apparatus*—Variations in rate of reflux, conditions of heating and time of extraction were examined. The suggested[3] time of 4 hours was shown to be insufficient in certain instances when ethanol alone was used as extracting solvent. Preliminary maceration with ethanol acidified with dilute acetic acid was therefore introduced.

(*ii*) *Extraction with trichloroethane*—When pure reserpine or rescinnamine was subjected to the method without the trichloroethane stage, the results were the same as those found when the full extraction procedure was used. It was therefore clear that none of the required alkaloids was lost at this stage. If the trichloroethane stage was omitted during a determination of rauwolfia itself, however, higher contents of apparent reserpine were obtained.

(*iii*) *Extraction with chloroform*—The Panel showed that solutions of reserpine in chloroforom deteriorate when exposed to daylight, a fall in extinction value of nearly 5 per cent. being noted within 1½ hours. It is therefore essential that this stage should be carried out as rapidly as possible and in subdued light.

(*iv*) *Evaporation of the chloroform*—The effect of removing the solvent at various temperatures under streams of air and nitrogen was examined. No significant differences between the methods were found.

(*v*) *Time of colour development*—An outside collaborator reported that he had obtained lower extinction values than had members of the Panel when preparing a calibration graph with pure reserpine. He proved that this was due to the lower temperature of his laboratory and suggested that the method of colour development proposed by Banes and others[3] might be more satisfactory. It was later confirmed by the Panel that the 3-hour development time was unsatisfactory for laboratory temperatures less than 22° C, and a change in procedure was adopted, whereby the final solution was heated at

55° C for 30 minutes to develop the colour. The effect on the slope of the calibration graph of developing the colour under various conditions is shown in Fig. 1. The importance of colour-development time and conditions in this reaction is still not appreciated by some workers; only recently, a time of only 1 hour at room temperature was prescribed.[22]

(*vi*) *Purity of trichloroethane used*—It was thought possible that some variation might be caused by the presence of chloroform as an impurity in the trichloroethane used. Gas-chromatographic examination of various samples used by the Panel showed chloroform to be absent.

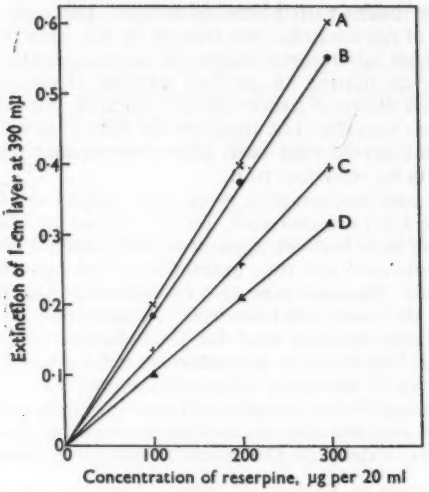


Fig. 1. Typical calibration graphs for reserpine when determined by the nitrite method: curve A, colour developed for 3 hours at 25° C; curve B, colour developed for 3 hours at 21° C; curve C, colour developed for 3 hours at 11° C; curve D, colour developed for 3 hours at 7° C. The graph obtained when colour is developed at 55° C for 30 minutes is identical with curve A

During examination of the above factors, inter-laboratory exchange of personnel and materials was made. The findings are incorporated in the proposed assay procedure for reserpine-type alkaloids in rauwolfia root and extracts (see Appendix I). When this method was applied to samples of rauwolfia and extracts, the inter-laboratory variation was considerably reduced. The results in Table II show a maximum spread of about $\pm$ 4 per cent. and a coefficient of variation, in the least satisfactory set of results, of 2·7 per cent. In the opinion of the Panel these results are acceptable, and it is unlikely that the reproducibility could be further improved. Table II includes some results for a sample of *R. mombasiana*, which occurs in Kenya and is thus a possible source of reserpine in British Overseas Territory.

During the examination of extraction conditions, a test was required for the detection of trace amounts of reserpine or rescinnamine. A study of many possible reactions, conveniently listed by Cheng Sun,[23] showed that the colour reaction with vanillin and hydrochloric acid was the most satisfactory. It was found to be sensitive down to 1 μg of pure reserpine or rescinnamine and to about 5 μg when drug extractives were also present. It is unlikely that this test would be required in the routine examination of rauwolfia, but details are given in Appendix II.

Results of collaborative tests

The extinction values found when the recommended procedure for colour development was applied to pure reserpine are shown in Table I. Table II shows the results found by the method recommended in Appendix I for reserpine-like alkaloids in samples of rauwolfia roots and extracts.

Table I

Extinction values for final coloured solutions of pure reserpine

Laboratory No.	Worker	Value of $E^{1\%}_{1cm}$ for solution containing— 100 μg of reserpine	200 μg of reserpine	300 μg of reserpine
1	A	409	407	404
	B	411	411	411
	C	414	414	413
	D	411	411	409
2	A	390	390	398
	B	392	393	394
3	A	403	403	402
	B	403	402	—
4	A	413	394	394
		391	391	395

Mean value found: 403.

Table II

Analysis of rauwolfia roots and extracts by the recommended method

Laboratory	Reserpine-like alkaloids found in— *R. serpentina* root 1, %	*R. serpentina* root 2, %	*R. vomitoria* root, %	*R. vomitoria* root bark 1, %	*R. vomitoria* root bark 2, %	*R. mombasiana* root bark %	Extract 1, %	Extract 2, %
A	0·170	0·194	0·353	1·20	1·02	1·74	6·59	22·6
	0·169	0·193	0·353	1·18	1·02	1·75	6·63	22·8
B	0·174	0·199	0·352	1·18	1·04	1·77	6·71	23·9
	0·174	0·197	0·351	1·19	1·04	1·77	6·64	23·9
C	0·171	0·204	0·346	1·18	1·00	1·77	6·72	23·3
	0·176	0·209	0·346	1·22	1·00	1·83	6·85	23·0
D	0·163	0·199	0·354	1·18	1·05	1·76	6·55	22·8
	0·163	0·199	0·357	1·19	1·02	1·76	6·55	23·0
Mean	0·170	0·199	0·351	1·19	1·02	1·77	6·65	23·1
Coefficient of variation ..	2·70	2·45	1·03	1·11	1·73	1·39	1·41	2·03

Conclusions

Methods for the determination of reserpine and similar alkaloids in rauwolfia have been examined. For routine examination of samples a procedure based on that described by Banes and his co-workers[3] is recommended. Certain modifications have been made to the method, and these have resulted in increased precision in collaborative trials.

The Panel wish to make grateful acknowledgement to Ciba Laboratories Ltd. and to Riker Laboratories Ltd. for gifts of pure alkaloids and of rauwolffa.

Appendix I

RECOMMENDED METHOD FOR THE DETERMINATION OF RESERPINE-LIKE ALKALOIDS IN RAUWOLFIA AND EXTRACTS

Principle of method—

After preliminary extraction of the root, the weakly basic alkaloids are separated and determined colorimetrically by a method based on their reaction with sodium nitrite and sulphuric acid.

APPLICABILITY—

The method is applicable to samples of *Rauwolfia serpentina*, *Rauwolfia vomitoria* and *Rauwolfia mombasiana* and to extracts from these species. It is probably equally applicable to many other species that have not been subjected to collaborative trial. Of the antihypertensive alkaloids, only reserpine and rescinnamine are determined; deserpidine gives no colour.

REAGENTS—

Ethanol, 95 per cent. v/v—Analytical-reagent grade.

Acetic acid solution—A 5 per cent. v/v solution of analytical-reagent grade glacial acetic acid in 95 per cent. v/v ethanol.

Sulphuric acid, dilute—An approximately 0·5 *N* solution of sulphuric acid in water.

Trichloroethane—1 : 1 : 1-Trichloroethane, obtainable from Kodak Ltd., Kirkby, Liverpool.

Chloroform—Analytical-reagent grade.

Sodium hydrogen carbonate solution—A 2 per cent. w/v aqueous solution of analytical-reagent grade sodium hydrogen carbonate. This solution should be prepared freshly as required.

Sodium nitrite solution—A 0·3 per cent. w/v aqueous solution of analytical-reagent grade sodium nitrite. This solution should be prepared freshly as required.

Sulphamic acid solution—A 5 per cent. w/v aqueous solution of sulphamic acid B.P. This solution should be prepared freshly as required.

Reserpine—To comply with the test described in the British Pharmacopoeia, 1959, p. 563, and the requirement that the value of $E^{1\%}_{1cm}$ for a standard solution prepared as described below and subjected to the recommended procedure for colour development should not be less than 390, calculated on the dried substance.

Standard solution of reserpine—Prepare a suitable amount of standard solution according to the following description. Dissolve 25·0 mg of reserpine by moistening with 2 ml of 95 per cent. ethanol and then adding 2 ml of 0·5 *N* sulphuric acid and finally 10 ml of ethanol. When warmed gently the reserpine readily dissolves. Cool the solution, and dilute to exactly 100 ml with 95 per cent. ethanol.

PROCEDURE—

The entire assay procedure should be carried out protected from the light as much as possible, and the alkaloids should be allowed to remain in chloroform for the minimum possible time. The use of grease as a lubricant for taps should be avoided.

Weigh accurately a suitable amount (see Note) of sample, ground to pass through a 60-mesh sieve, and triturate it with 10 ml of acetic acid solution. Set the mixture aside for 2 hours, stirring from time to time. Transfer the mixture (with ethanol) to a Soxhlet apparatus, and extract for 4 hours with ethanol. During extraction, the apparatus should be shielded from the light. Transfer the cooled extract, concentrated if necessary, to a 100-ml calibrated flask, and dilute to 100 ml with ethanol.

Transfer 20 ml of the extract to a separating funnel containing 200 ml of dilute sulphuric acid, mix, and extract with 3 portions, each of 25 ml, of trichloroethane. Wash each of the trichloroethane extracts in a second separating funnel containing 50 ml of dilute sulphuric acid, and discard the organic layer. Extract the weakly basic alkaloids from the aqueous acid solution by shaking with 20 ml and then with 5 portions, each of 15 ml, of chloroform. Wash each chloroform extract with the acid in the second separating funnel and then with 2 portions, each of 10 ml, of sodium hydrogen carbonate solution in two additional separating funnels. Filter the chloroform extracts through a small plug of cotton-wool into a 100-ml calibrated flask, dilute to the mark with chloroform, and mix thoroughly.

Transfer duplicate 20-ml portions of the solution to boiling tubes, and evaporate to dryness on a water bath in a current of warm air, protecting the tubes from the light. To the contents of each tube add 10 ml of ethanol and 2 ml of dilute sulphuric acid, and warm to dissolve the residue.

In one tube (the test solution) place 2 ml of sodium nitrite solution. Mix each of the solutions, and heat the tubes in a water bath at 55° C for 30 minutes, protecting the solutions from the light. Cool, place in each tube 1·0 ml of sulphamic acid solution, and transfer the

contents to a 20-ml calibrated flask. Rinse the tube with ethanol, used in small portions, and use the rinsings to adjust the volume to 20 ml.

Determine the extinction at 390 mμ of a 1-cm layer of the test solution, using the second solution (without nitrite added) in the comparison cell.

Compare the value obtained* with a calibration graph prepared as described below at the same time as the test, and hence calculate the percentage of reserpine-like alkaloids in the sample.

NOTE—For whole-root samples of *R. serpentina* and *R. vomitoria*, which contain between 0·10 and 0·40 per cent. of reserpine-like alkaloids, use 2·5 g.

For root-bark samples of *R. vomitoria*, which may contain more than 1 per cent. of reserpine-like alkaloids, use 1·0 g. If the extinction values of the final coloured solutions are too great, test smaller aliquots of the chloroform solution, without delay, or extract a smaller portion of the ethanolic solution with trichloroethane.

For extracts of rauwolfia, dissolve a suitable amount, dependent on the concentration, in 100 ml of ethanol, and continue as described above, beginning at "Transfer 20 ml of the extract to a separating funnel. . . ."

PREPARATION OF CALIBRATION GRAPH—

By suitable dilution of the standard solution of reserpine prepare standards containing 100, 200 and 300 μg of reserpine in 10 ml of ethanol.

By pipette, measure 10 ml of each of these solutions into boiling tubes, and add to each 2 ml of dilute sulphuric acid and 2 ml of sodium nitrite solution. Mix the solutions, and heat them in a water bath at 55° C for 30 minutes, protecting the tubes from light. Cool, place 1·0 ml of sulphamic acid solution in each tube, and transfer the contents to a 20-ml calibrated flask. Rinse the tube with ethanol, used in small portions, and use the rinsings to adjust the volume to 20 ml.

Determine the extinction of a 1-cm layer of each solution at 390 mμ, using in the comparison cell a blank solution prepared in a similar manner, but containing 10 ml of ethanol instead of the standard solution of reserpine.

Plot a graph of extinction against concentration of reserpine present.

Appendix II

QUALITATIVE TEST FOR THE DETECTION OF TRACE AMOUNTS OF RESERPINE OR RESCINNAMINE

REAGENT—

Vanillin solution—A 1 per cent. w/v solution of vanillin in hydrochloric acid, sp.gr. 1·18. This solution should be prepared immediately before use.

PROCEDURE—

Evaporate a portion of the solution suspected to contain the alkaloid in a small porcelain dish by warming on a water bath until almost dry, and complete the evaporation at room temperature. Add 2 to 3 drops of vanillin solution, and mix with the residue by rubbing with a glass rod. A pink colour develops within 2 minutes if reserpine or rescinnamine is present.

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Notes

RAPID MICROANALYTICAL METHOD FOR DETERMINING CARBON AND HYDROGEN IN FLUORO-ORGANIC COMPOUNDS

CERTAIN types of compound are known to give low carbon and hydrogen values by the slow Pregl method; for this reason the rapid method proposed by Belcher and Ingram[1] is used for all determinations in this laboratory. As the determination of carbon and hydrogen in fluorine-containing compounds by rapid combustion does not appear to have been dealt with in the literature, a method based on that of Belcher and Goulden[2] has been developed. Magnesium oxide has been used by Throckmorton and Hutton[3] and McCoy and Bastin[4] as an absorbent for the silicon tetrafluoride formed during combustion. However, Rush, Cruikshank and Rhodes[5] found that magnesium oxide became inactive after a few determinations owing to overheating or exhaustion. They therefore proposed the use of magnesium aluminate together with a layer of lead dioxide. The objections to the use of lead dioxide, as found by McCoy and Bastin and many other workers, are that the results for hydrogen are unreliable owing to dehydration of this particular absorbent; I have confirmed this effect while using the normal Pregl method for determinations of carbon and hydrogen. Belcher and Goulden used sodium fluoride packed into the combustion tube with successful results, the analysis being carried out according to the well known Friedrich method. The sodium fluoride packing is easily prepared, the operating temperature is relatively low, and the capacity for absorbing silicon tetrafluoride is good; the investigation was therefore carried out with sodium fluoride as an absorbent.

METHOD

A paste of analytical-reagent grade sodium fluoride was made with distilled water and baked at 110° C. The cake was then made into pellets, which were passed through a sieve, the portion between 10 and 14 mesh being retained for use.

To avoid disturbing the main combustion tube, a glass tube was constructed and packed with the prepared sodium fluoride. The tube was of Pyrex glass, 11 mm external diameter and 190 mm long, which included a beak, 3·5 mm external diameter and 30 mm long, and a B7 socket at the opposite end. The stopper was a B7 cone with the end drawn out to a tube, 3·5 mm external diameter, of total length 30 mm. Lugs were attached to both the tube and stopper, which were then held firmly together by steel springs. The beak end of the tube was packed with a plug of silver wool 5 mm long and a platinum wire along the length of the beak to prevent the condensation of water. The prepared sodium fluoride was then packed into the tube to a length of 120 mm, the remaining space in the tube up to the joint being packed with silver wool. The cone and tube of the stopper were completely filled with silver wool, which conducted sufficient heat to prevent condensation of water. An auxiliary furnace was constructed from a piece of 2-inch diameter aluminium rod 140 mm long, a centre hole being bored for the sodium fluoride tube and a smaller hole below and parallel to the centre hole for an electrical heating element. The tube was attached

to the outlet of the main combustion tube and the furnace was placed in position, the rest of the train then being assembled in the ordinary way. The sodium fluoride was heated to 270° ± 10° C; a single filling of the tube will last for approximately 200 determinations. The normal procedure for determining carbon and hydrogen was then carried out, halogens and sulphur, if present, being absorbed on the normal silver packing of the main combustion tube.

Results

The results are accurate to within ±0·2 per cent. for both carbon and hydrogen (see Table I). Many types of compounds have been analysed, including those containing, besides carbon, hydrogen and fluorine, oxygen, nitrogen, chlorine, bromine and sulphur. Fluorocarbons have not been analysed, but should not present any difficulties if a weighed amount of benzoic acid is added to the weighed sample.

Seventy analyses have been carried out without visible sign of deterioration of the silica combustion tube. The rapid flow rate will carry the hydrogen fluoride formed along the tube for a few millimetres before it comes into contact with the silica walls of the tube, and by this time it will have reached the silica-wool plug placed in the combustion tube at the point of entry into the main furnace. All, or nearly all, of the reaction between the hydrogen fluoride and the silica will take place here, thereby avoiding any damage to the tube.

Table I

Determination of carbon and hydrogen in some fluoro-organic compounds

Compound	Carbon calculated, %	Carbon found, %	Hydrogen calculated, %	Hydrogen found, %
p-Fluorobenzoic acid	60·00	60·15	3·60	3·50
Trifluoro-acetanilide	50·80	50·74	3·20	3·10
m-Trifluoromethylbenzoic acid	50·54	50·74	2·65	2·70
Research compounds	58·60	58·56	6·80	6·75
	72·83	72·81	7·80	7·95
	61·77	61·66	6·00	5·88
	65·90	65·95	6·08	6·00

Conclusions

The proposed method gives satisfactory results that are within the accepted limits of accuracy for microanalytical work. It permits analyses of fluoro-organic compounds to be carried out rapidly and removes the necessity of having to prepare a separate combustion tube, together with the inconvenience of setting up and conditioning that tube each time such a compound is submitted for analysis. With the proposed method analyses can be carried out immediately after the sodium fluoride tube has been connected, the furnace has been brought up to temperature and the procedure has been carried out on one unweighed sample to condition the sodium fluoride. Thus, little time is lost and the normal set-up for determining carbon and hydrogen is not disturbed in any way; the main combustion tube, not having been attacked, lasts for its normal working life.

I thank the Directors of Pfizer Ltd. for permission to publish this Note.

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THE DETECTION OF ELEMENTARY SULPHUR BY MEANS OF ACETONE

ELEMENTARY sulphur can be detected by converting it to thiocyanate[1] or thiosulphate,[2] but a convenient and sensitive test, applicable when thiocyanate or thiosulphate ions (or both) are present initially, has been lacking hitherto. We have now developed such a test, although it cannot be used when the specimen contains polysulphide.

The test relies on a striking colour reaction, which was first described by Gil[3] and subsequently investigated by a number of workers.[4] Briefly, a characteristic bluish colour ("blue sulphur") is produced when sodium or potassium polysulphide is treated with various organic liquids and is particularly intense when acetone is used. This affords a method of detecting sulphur, as this element readily combines with sodium sulphide (which does not give a bluish colour with acetone) in aqueous or ethanolic solution to produce sodium polysulphide.

METHOD

REAGENTS—

Sodium sulphide solution, 2 per cent. w/v, aqueous—Prepare from polysulphide-free material.
Acetone—Analytical-reagent grade.
Ethanol—Absolute alcohol B.P.

PROCEDURE—

Place 1 drop of the test solution in a 1-ml porcelain crucible, add 1 drop of sodium sulphide solution, and then immediately add 0·5 ml of acetone. The production of a blue or greenish blue colour denotes that the specimen contained elementary sulphur. The colour produced varies with the concentration of sulphur; when this is sufficiently low, the reaction mixture becomes sky-blue, but, with larger concentrations of sulphur, a greenish tinge is introduced because the acetone causes a yellow oil to separate.

For a solid mixture containing particles of sulphur, place a few milligrams of the powdered material in the crucible, and extract with 3 or 4 drops of ethanol, warming if necessary and replenishing with ethanol as required. Add 1 drop of sodium sulphide solution and then 0·5 ml of acetone to the cooled extract.

The sensitivity of the test was determined by progressively diluting a solution containing 0·06 g of sulphur in 100 ml of ethanol with ethanol. The lower limit of identification was 6 μg of sulphur, and the concentration limit was 1 in 30,000.

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A TEST FOR THE DETECTION OF AROMATIC AMINES

A COLOUR test for aromatic amines has been developed as a result of an observation made when attempting to detect alcohols with ammonium ceric nitrate reagent solution. Instead of the expected faint red colour, an intense blue-black precipitate was obtained, and further tests revealed that the interfering substance was aniline. Although this interference has been reported,[1] it is believed that use has not previously been made of this information to detect or identify aromatic amines.

Ceric salts, conveniently in the form of a solution of ammonium ceric nitrate in dilute nitric acid, can produce highly coloured oxidation products with aromatic amines dissolved in a variety of organic solvents. In general, the colours change, often distinctively, when the reaction mixture is warmed. The phenomenon appears to be of use for the characterisation of aromatic amines;

thirty-three such amines were found to respond to the reaction, whereas no aliphatic or alicyclic amine or other nitrogen-containing compound tested gave any coloured product under the conditions used.

The reproducible development of the colour, necessary for test purposes, is dependent on the relative amounts of solvent, ceric nitrate reagent and amine present. For routine purposes, satisfactory conditions are established by carrying out the test as described below.

Dissolve 0·05 g of the free base or its approximate equivalent weight as an amine salt in 100 ml of acetone - water mixture (3 + 1 by volume). To 10 ml of this solution add 2 drops only of a solution prepared by dissolving 10 g of ammonium ceric nitrate in 100 ml of 5 per cent. nitric acid. Mix, and note the colour. Warm the mixture in a water bath for 30 seconds to 1 minute, and note the final colour.

Results

Table I shows the colours obtained under these experimental conditions for a number of primary, secondary and tertiary aromatic amines.

Table I

Colours obtained with various aromatic amines

Amine	Initial colour	Final colour
Aniline	Purple	Grey-blue
o-Toluidine	Violet	Blue-grey to green-grey
m-Toluidine	Magenta	Magenta
p-Toluidine	Pale red	Red-orange to red
2:3-Xylidine	Violet	Violet
2:4-Xylidine	Very pale pink	Very pale pink
2:5-Xylidine	Magenta or violet-red	Pale magenta
2:6-Xylidine	Green	Very pale pink
3:4-Xylidine	Pale purple	Very pale pink
o-Chloroaniline	Red-violet	Red
p-Chloroaniline	Bright red	Bright red
m-Chloroaniline	Red	Pale red
p-Bromoaniline	Bright red	Bright red
p-Iodoaniline	Bright red	Bright red
o-Aminophenol	Orange-brown	Yellow
m-Aminophenol	Pale orange-brown	Very pale brown
p-Aminophenol	Very pale pink	Very pale pink
Orthanilic acid	Violet	Pale orange-brown
Metanilic acid	Purple	Pale grey
Sulphanilic acid	Bright red	Pale mauve to red
Anthranilic acid	Very light brown	Very light brown
p-Aminobenzoic acid	Pale red	Light brown
Benzidine	Emerald green	Pale red
o-Tolidine	Emerald green	Khaki
1-Naphthylamine	Violet	Crimson
2-Naphthylamine	Green	Amber to brown
o-Phenylenediamine	Yellow	Yellow
m-Phenylenediamine	Brown	Pale brown
p-Phenylenediamine	Light olive-green	Brown to purple
N-Monomethylaniline	Olive-green	Blue
NN-Dimethylaniline	Golden yellow	Yellowish green
Diphenylamine	Very deep purple	Pale lilac
Triphenylamine	Very deep purple	Violet-grey

Nuclear-substituted nitro-derivatives of aromatic amines do not respond to the test, except to show an enhancement of their natural yellow colour; N-acetyl- or N-benzoyl-derivatives of aromatic amines do not respond.

The following nitrogen-containing compounds were tested and gave no reaction under the conditions stated: methylamine, trimethylamine, triethylamine, *n*-butylamine, di-*n*-butylamine, triethanolamine, *cyclo*hexylamine, di*cyclo*hexylamine, pyridine, piperidine, quinoline, 2-, 3- and 4-methylpyridine and 2-, 3- and 4-aminopyridine.

The test serves to distinguish aromatic amines from other nitrogen-containing compounds. It can also be of use to characterise a particular amine, sometimes, when the test is not wholly distinctive, in conjunction with some additional differentiating test. It may be of especial value for distinguishing between isomers (see, for example, the colour reactions of the three toluidines in Table I).

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THE DETERMINATION IN VINYL ESTERS OF MERCURY DERIVED FROM MERCURIC ACETATE CATALYST

THE manufacture of vinyl esters entails the use of mercuric acetate in catalytic amounts.[1] The problem of determining residual amounts of mercury in the final product did not yield to a variety of techniques[2 to 9] and led to the development of a method of direct precipitation and determination of the metal at levels of 1 to 100 p.p.m. in organic solution by means of hydrogen sulphide.

The mercury determined in actual experiments was principally in a complex form and not as mercuric acetate. The concentrations detected by the method were confirmed by emission spectroscopy down to a level of 15 p.p.m. Flame photometry was also used, but proved to be limited to concentrations of 200 p.p.m. of mercury or more.

The method was applied with equal success to the vinyl esters of butyric, valeric, caproic, caprylic, pelargonic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic and benzoic acids.

METHOD

In a three-necked round-bottomed 1-litre flask, equipped with stirrer, condenser and gas-bubbler, place 15 ml of nitrobenzene, and thoroughly wet the walls of the flask. Add 250 g of sample (vinyl acetate or solution of higher esters), 6 ml of glacial acetic acid and 9 ml of 2 *N* hydrochloric acid, and then pass hydrogen sulphide through the solution with rapid stirring for 45 minutes; 15 minutes through the cold solution (to fix organo-mercurials), 15 minutes while the temperature is being raised to boiling-point on a water bath and, finally, 15 minutes through the solution boiling under reflux.

Stop the flow of gas, turn off the heating, add 250 ml of boiling ethanol containing 10 ml of nitrobenzene, and stir for a further 5 minutes. Stop the stirrer, dismantle the apparatus, and filter the solution through a tared No. 4 sintered-glass crucible as rapidly as possible at the pump. Wash the precipitate under gravity (*a*) with 80 to 100 ml of boiling ethanol to remove organic materials and sulphur, (*b*) with 40 to 50 ml of carbon disulphide to remove sulphur and (*c*) with 40 to 50 ml of ether to assist drying.

Suck the precipitate dry, and complete the drying in an air-oven at 110° to 120° C to constant weight. Occasionally it is necessary to repeat the washing cycle before constant-weight conditions can be attained. The whole determination can usually be completed within 90 minutes.

NOTES ON THE METHOD

1. Conventional coagulants for sulphide precipitates were found to be ineffectual, but nitrobenzene (personal communication from B. J. Green) proved to be an efficient coagulant for mercuric sulphide; in addition, it reduces the tendency for the precipitate to adhere to the glass.

2. The addition of boiling ethanol rendered soluble any colloidal sulphur present, thus preventing clogging of the filter.

3. Acid conditions are required for complete precipitation of the mercury; the proportions of acetic and hydrochloric acids quoted were found to meet these requirements in these particular systems without introducing the difficulties that arise through non-homogeneity.

4. Blank tests on the vinyl acetate used for dilution and control experiments yielded zero figures for mercury contents.

5. Control experiments on vinyl acetate containing 5 and 25 p.p.m. of mercury (as mercuric acetate) gave results of 5·5 and 5·0 and 25·0 and 24·5 p.p.m., respectively. Subsequent results have always been quoted to the nearest 1 p.p.m.

6. Certain organo-mercury compounds, *e.g.*, sodium ethyl mercury thiosalicylate and sodium *p*-ethyl mercuric thiophenyl sulphonate were examined, but did not give a precipitate.

Results

The results in Table I were obtained in a sequence of 150 consecutive duplicate determinations during the first 6 months of production control.

Table I

Analysis of results

Deviation of duplicate results, p.p.m.	Number of pairs in quoted limit	Minimum mercury level, p.p.m.	Maximum mercury level, p.p.m.
0	48	1	110
1	72	1	275
2	16	2	70
3	7	6	85
4	2	95	280
5	4	10	100
6	1	270	276
>6	—	—	—

From these results it is apparent that the method is not only reliable and reproducible, but also has a considerable validity for mercury contents of the order of 1 to 10 p.p.m.

We thank the Directors of Vinyl Products Limited for permission to publish this Note and M. Harrison for his assistance with the experimental work.

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THE DETERMINATION OF ZINC IN INDIUM AND NICKEL

The determination of small amounts of zinc in indium used for semi-conductor alloys has been investigated. The usual method advocated for separating zinc from indium comprises precipitation of indium by ammonia in presence of ammonium chloride to leave zinc in solution. By this procedure, in spite of repeated re-precipitation of indium hydroxide, recoveries of zinc were invariably low; the use of sodium hydroxide instead of ammonia resulted in even lower recovery.

Bishop and Liebmann's[1] method for determining zinc in lead - tin alloys has been found to be adaptable to the determination of zinc in indium and also in grades of nickel used for electronic purposes.

Method

Apparatus—

Chromatographic columns fitted with sintered-glass discs and exit taps leading into 250-ml Buchner flasks (Quickfit & Quartz Ltd., Nos. CR32/20 and CR3T/23, fitted with B24 joint) were used for the elution.

A Cambridge polarograph with a Univector attachment was used for recording the polarograms.

Reagents—

Unless otherwise stated, all materials should be of analytical-reagent grade.

Ferric chloride solution—Prepare a solution containing 1 mg of iron per ml, and purify by extracting with a 0·01 per cent. solution of dithizone.[1]

n-*Butyl alcohol.*

Hydrochloric acid, diluted (1 + 1)—Mix equal volumes of distilled water and hydrochloric acid, sp.gr. 1·18.

Perchloric acid, 60 to 70 per cent.

Ethanol, absolute.

n-*Butyl alcohol - hydrochloric acid mixture*—Mix 960 ml of *n*-butyl alcohol and 40 ml of hydrochloric acid diluted (1 + 1).

Cellulose powder—Whatman standard grade, ashless.

Base electrolyte solution—Prepare a solution 0·2 *M* in ammonium chloride, *M* in ammonia, 0·1 *M* in sodium sulphite and containing 0·005 per cent. of gelatin.

PROCEDURE—

Determination of zinc in indium—Dissolve 1 g of sample in 20 ml of hydrochloric acid (1 + 1), and dilute the solution with water to 250 ml in a calibrated flask. By pipette, place an aliquot containing 10 to 50 μg of zinc in a small beaker, add 1 ml of ferric chloride solution, and evaporate to dryness on a water bath. Dissolve the residue in the minimum amount of hydrochloric acid, add 0·5 ml of *n*-butyl alcohol, transfer to a column of cellulose (approximately 6 inches deep and ¾ inch in diameter, previously washed with 100 ml of *n*-butyl alcohol - hydrochloric acid mixture to remove any zinc), and elute with *n*-butyl alcohol - hydrochloric acid mixture. Continue elution until the yellow band due to iron has been completely eluted, when all the zinc will be in the eluate; approximately 100 ml of eluting mixture will be required. Transfer the eluate to a 150-ml beaker. Rinse the receiver with 15 ml of absolute ethanol and then with 7 ml of water, and add the rinsings to the main solution. Evaporate the combined solution to small volume, transfer to a 10-ml beaker, and evaporate to dryness. Add 2 ml of perchloric acid, and again evaporate to dryness. Dissolve the residue in 5 drops of hydrochloric acid (1 + 1), and transfer the solution to a centrifuge tube graduated at 5 and 10 ml. Make the solution slightly ammoniacal, dilute to 5 ml, and add 5 ml of base electrolyte solution. Mix thoroughly, spin in a centrifuge, and decant the supernatant solution from the ferric hydroxide into a polarographic cell. Record a polarogram between −1·0 and −1·5 volts against a mercury-pool anode, and determine zinc by reference to standard polarograms.

Determination of zinc in nickel—Dissolve 0·5 g of nickel in hydrochloric acid (1 + 1), add 10 mg of cobalt, as cobalt chloride, and 1 ml of ferric chloride solution, and continue exactly as described above. Cobalt, during elution, follows iron down the column as a blue band and seems to act as a barrier to the nickel, which otherwise has a tendency to "trail."

When 50 μg of zinc were added to a portion of the base electrolyte solution, and a polarogram was recorded with the instrument at half maximum sensitivity, the peak height was 40 mm. Eight solutions prepared from zinc-free nickel to which 50 μg of zinc had been added were treated by the proposed procedure; the peak heights found were 40, 40, 39, 40, 39, 40, 39 and 41 mm. A blank solution is normally subjected to the entire procedure with each batch of determinations; polarograms for such solutions usually show a peak about 3 to 4 mm in height, this value being deducted from the assay readings.

REFERENCE

1. Bishop, J. R., and Liebmann, H., *Analyst*, 1953, **78**, 117.

JOHNSON, MATTHEY & CO. LTD.
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EXHIBITION GROUNDS,
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E. G. TOWNDROW
R. HUTCHINSON
H. W. WEBB

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Apparatus

A MODIFIED APPARATUS FOR THE MICRO-DETERMINATION OF MOISTURE IN ORGANIC COMPOUNDS

THE requirement was for a simple apparatus for determining moisture on the micro scale; it had to be small enough to be easily transferred to and from a dry box, as most samples, when dry, are hygroscopic and electrostatic and need special handling. Dried samples are often so hygroscopic that, in withdrawing the weighing bottle from an ordinary drying "pistol," they pick up moisture before the bottle can be stoppered. For normal drying or determination of moisture on the macro scale, this small amount of moisture can be ignored, but on the micro scale it is significant. This error must be overcome before an accurate determination can be made.

Moisture is normally removed from a sample in two ways. In one, dry nitrogen is passed over the sample, which is heated to a suitable temperature; in the other, the sample container, heated at the required temperature, is evacuated over phosphorus pentoxide.

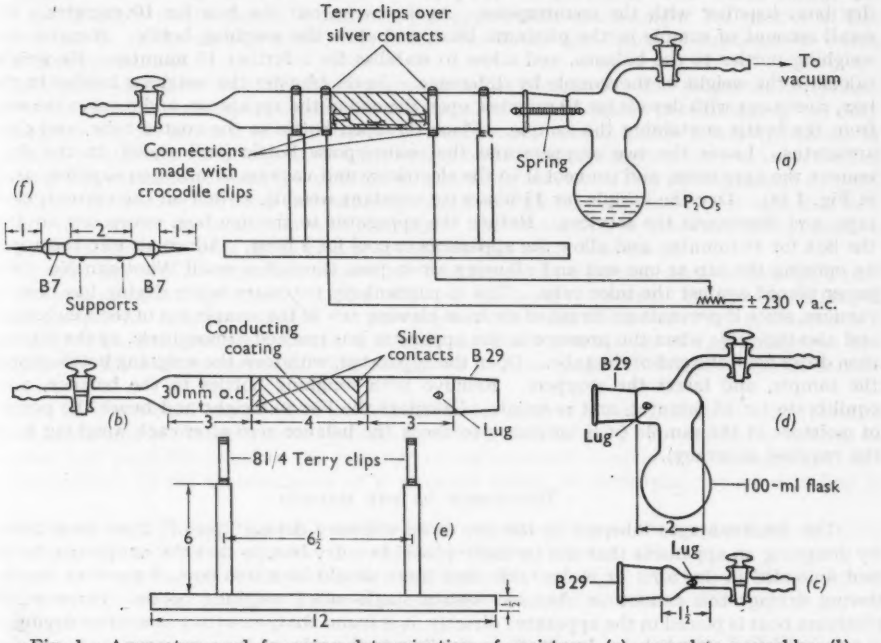

Fig. 1. Apparatus used for micro-determination of moisture: (*a*), complete assembly; (*b*) coated Pyrex-glass tube; (*c*) B29 socket fitted with tap; (*d*) 100-ml flask fitted with tap and B29 socket; (*e*) stand; (*f*) double-ended weighing bottle. Except where otherwise stated, all dimensions are in inches

DESCRIPTION OF APPARATUS

The apparatus is designed to be easily converted for either method of drying. Use is made of a Pyrex-glass tube having an electrically conducting coating to obviate the necessity of winding a heating coil. Such tubes can be supplied to the specification required and can be operated to a maximum temperature of 350° C.

The complete assembly is shown in Fig. 1 (*a*) and consists of four parts: the coated tube fitted with a tap at one end and a B29 cone at the other, shown in Fig. 1 (*b*); a B29 socket fitted with a tap, shown in Fig. 1 (*c*); a 100-ml flask fitted with a tap and a B29 socket, shown in Fig. 1 (*d*); and a stand consisting of a base-board, 12 inches × 4 inches × ½ inch, on which are fitted two uprights of Tufnol rod, ¾ inch in diameter. A Terry clip (No. 81/4) is screwed to the top of each upright to support the coated tube.

The arrangement shown in Fig. 1 (*c*) is used when the sample is to be dried in a current of nitrogen, and that shown in Fig. 1 (*d*) is used with phosphorus pentoxide in the flask when drying under vacuum. All joints are fitted with lugs so that they can be held together with springs.

Electrical contact is made through Terry clips that fit over silver contacts on the tube, wires being attached by means of crocodile clips. A sliding resistor is used to vary the temperature and is calibrated so that it can be pre-set to give any required temperature.

The sample boat is contained in a double-ended weighing bottle, shown in Fig. 1 (*f*), to allow through passage of gas during drying. The weighing bottle is made with B7 joints so that the stoppers are interchangeable.

Method of operation

Place a platinum boat, two weighing bottles (the lighter to act as a counterpoise) and the drying apparatus in a dry box. Remove the stoppers from the weighing bottles, open the drying apparatus, and sweep dry air through the box for 10 minutes. Place the platinum boat in the heavier weighing bottle, insert stoppers in both bottles, remove them to a microbalance, and allow to equilibrate for 15 minutes. Weigh the empty boat and the bottle together, and transfer to the dry box, together with the counterpoise. Again sweep out the box for 10 minutes. Place a small amount of sample in the platinum boat, and close the weighing bottle. Remove the two weighing bottles to the balance, and allow to stabilise for a further 15 minutes. Re-weigh, and calculate the weight of the sample by difference. Again transfer the weighing bottles to the dry box, sweep out with dry air for 10 minutes, open the end of the apparatus, and remove the stoppers from the bottle containing the sample. Place the open bottle in the coated tube, and close the apparatus. Leave the two stoppers and the counterpoise bottle (still sealed) in the dry box, remove the apparatus, and connect it to the electricity and vacuum or nitrogen supplies, as shown in Fig. 1 (*a*). Dry the sample for 1½ hours (to constant weight), switch off the current, close the taps, and disconnect the services. Return the apparatus to the dry box, sweep dry air through the box for 10 minutes, and allow the apparatus to cool for 1 hour. Admit air into the apparatus by opening the tap at one end and allowing air to pass through a small Whatman No. 50 filter-paper placed against the inlet tube. This is particularly necessary when drying has been under vacuum, since it prevents an inrush of air from blowing any of the sample out of the weighing bottle and also indicates when the pressure in the apparatus has reached atmospheric, as the filter-paper then drops from the end of the tube. Open the apparatus, withdraw the weighing bottle containing the sample, and insert the stoppers. Remove both weighing bottles to the balance, allow to equilibrate for 15 minutes, and re-weigh. Calculate the loss in weight and hence the percentage of moisture in the sample (it is necessary to check the balance zero after each weighing to attain the required accuracy).

Discussion of the method

The disadvantages inherent in the use of an ordinary drying "pistol" have been overcome by designing an apparatus that can be easily placed in a dry box, so that the sample can be loaded and unloaded in dry air. It is desirable that there should be a free flow of gas over the sample during drying; this cannot be obtained with a single-ended weighing bottle. However, if the platinum boat is placed in the apparatus directly, it is found that, on withdrawal after drying, there is a grave risk of sample being lost by "jumping" on to the sides of the apparatus (owing to electrostatic attraction). This has been overcome by using a double-ended weighing bottle, which permits the sample to be left in the weighing bottle and yet allows free flow of gas over it. When the weighing bottle is withdrawn from the apparatus, any electrostatic "jumping" occurs only on to the walls of the weighing bottle, and the weight of the sample is not affected.

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A. C. Thomas
Received *April 1st*, 1960

Book Reviews

ORGANOSILICON COMPOUNDS. By C. EABORN, Ph.D., D.Sc. Pp. x + 530. London: Butterworths Scientific Publications. 1960. Price 80s.

An understanding of the chemistry of organosilicon compounds is necessary to their intelligent use, and, in as much as the chemistry of these compounds is not ordinarily treated in our text-books, it is fortunate that a compact and comprehensive survey of our present knowledge in this field is now available. This book should be of value to the prospective research student in organosilicon chemistry and to those involved in industrial applications of organosilicon compounds.

The contents may be conveniently considered in three parts for the purpose of a review. The first deals essentially with the formation and reaction of silicon and carbon bonds, together with certain aspects of organosilicon chemistry, such as the stereochemistry of quadrivalent silicon, electronegativities, ionic character of bonds and bond energies. The mechanism of substitution at the silicon atoms is discussed; the choice of words, such as "backside" and "flank attack," to explain the possible mechanism of substitution is appropriate and brought the picture of the possible mode of mechanism to mind very well. However, the choice of explanatory words could be more consistent.

The second part gives an exhaustive treatment of the preparation and properties of specific classes of compounds, such as the organosilicon halides, hydrides, silanols and siloxanes, but only an elementary outline of the industrial aspects of silicones is included. This is mainly because in the author's own words "it is impossible for someone outside the silicone industry to know the full facts about the manufacture and composition of silicone products." This short account is, however, quite well done, apart from the paragraph dealing with silicone greases. It is stated that "they are valuable for use in contact with solvents." This cannot be so, since the methyl silicone oils, which constitute the bulk of the grease, are soluble in a range of solvents.

The third part deals with some aspects of their physical properties, such as density, molar refraction and molecular spectrum. Tables of group and bond refractions, ultra-violet, infra-red and Raman spectral data are included. A few pages are devoted to analysis; this is essentially a review of published analytical methods, and little comment is made on the advantages or disadvantages of the various methods. The usefulness of these pages is limited to the references supplied.

There is, however, a good deal of potentially useful analytical information in other parts of the book, and some of the methods of cleavage have been used to carry out qualitative analysis on unknowns, *e.g.*, page 126 refers to the digestion of organosilicon compounds with sulphuric acid and identification of the evolved gases as a proposed means of identifying groups attached to silicon.

All the bibliographies in this book are good and comprehensive, so that, apart from the value of the articles themselves, they provide an invaluable source of recent references.

Those of us interested in organosilicon compounds owe a debt of gratitude to the author of this book for producing such a comprehensive, well indexed and tabulated account of the present knowledge of organosilicon chemistry.

J. C. B. SMITH

CHROMATOGRAPHIE EN CHIMIE ORGANIQUE ET BIOLOGIQUE. VOLUME I. GÉNÉRALITÉS; APPLICATIONS EN CHIMIE ORGANIQUE. Edited by E. LEDERER. Pp. xiv + 671. Paris: Masson et Cie. 1959. Price (paper) 90 NF.; (cloth boards) 100 NF.

The declared aim of this book is to put at the disposal of chemists a complete text that allows them to do the greater part of chromatography without consulting the original texts. It cannot be said to have achieved this aim, if only because in such a rapidly advancing subject a text-book is almost inevitably out of date when published. The bibliographies are excellent up to 1956, but references for 1957 are few, and still fewer are later. This is particularly unfortunate with gas chromatography in that the modern highly sensitive detectors and capillary columns are not mentioned and McWilliam, Lovelock, and Golay do not occur in the author index.

The first part of the book is devoted to separate sections on absorbtion, ion-exchange, partition and paper and gas chromatography and radio-isotopic techniques by different authors. The theory of each section is separately discussed, so that there is much overlapping. The subject

would seem much simpler if a consistent nomenclature throughout the different articles had been adopted and if, indeed, a more critical approach had been used. The section on partition and paper chromatography is perhaps the best and comes nearest to meeting the declared aims. In the ion-exchange section, Moore and Stein's work on amino acid separation is not discussed at all and the effects of cross-linking are discussed in a third of a page. The section on radio-isotopic techniques deals mostly with paper chromatography. Identification and isotope-dilution methods are discussed, but no adequate discussion of counting errors is given, and safety precautions are not mentioned.

The second part of the book deals with the separation by any appropriate chromatographic method of various groups of compounds, hydrocarbons, mono and polyhydric alcohols, phenols, aldehydes, ketones, acids, nitro compounds, volatile amines, alkaloids, halogen compounds, synthetic dyestuffs and stereoisomers. It is a little surprising that hydrocarbons have only 25 pages, compared with 47 for acids. The last article, on stereoisomers, assembles a large amount of data not previously brought together.

A. J. P. MARTIN

CHROMATOGRAPHIE EN CHIMIE ORGANIQUE ET BIOLOGIQUE. VOLUME II. APPLICATIONS EN CHIMIE BIOLOGIQUE. Edited by E. LEDERER. Pp. xvi + 876. Paris: Masson et Cie. 1960. Price (paper) 130 NF; (cloth boards) 140 NF.

This volume is the second part of a monograph on chromatography, under the editorship of E. Lederer. The first part, published in 1959, treated the theory of chromatography and its applications in organic chemistry. This part deals with its applications to biological chemistry. In the preface, the editor gives as his aim the presentation of chromatographic procedures in sufficient detail and with special selection of really effective methods to enable the chemist "to carry out chromatography without consulting the original literature." This should perhaps not be taken too literally, especially in view of the excellent bibliographies throughout the book. The editor has certainly achieved the production of a well documented compendium on the chromatography of natural products.

The book is arranged according to the various groups of natural products, and each chapter is by a specialist in this field. Each writer has selected the well established methods and describes them in detail, mentioning only briefly the historical aspect of his subject.

Adsorption chromatography, ion-exchange techniques and partition chromatography are described, in this order, in each chapter, both for column and paper chromatography. Methods of detection and identification follow. This arrangement makes for clarity of description and permits the reader to find rapidly the method best suited to his purpose.

The literature is covered up to 1957 and several addenda bring the subject matter up to 1958.

In the first chapter, on sugars, R. Dedonder describes adsorption on charcoal, partition of sugar derivatives on silica gel and the chromatography of sugars on powdered cellulose and on paper. A welcome feature in this part, as well as of the following chapters, is the inclusion of numerous tables giving R_F values or retention values under well defined conditions, colour reactions, etc. These tables will be a great help for the identification of unknown substances and for the selection of analytical methods.

Phosphoric esters are described by J. Montreuil, and sulphur compounds by P. Fromageot. In the chapter on sterols and steroids, by R. Neher, the connection between structure and R_F value is brought out well. The chapter on lipids, by J. Asselineau, shows the complexity of the subject and the need for much further work. M. Juticz describes amino alcohols in a short chapter, and amino acids and peptides, followed by proteins, in some well written chapters that do justice to the amount and quality of work accomplished in this field.

Among the subsequent chapters, that by P. Boulanger and J. Montreuil, on nucleic acids and their products of varying degrees of degradation, should be mentioned for its wealth of detail. Shorter chapters on pigments, fat- and water-soluble vitamins, growth factors, protein hormones and antibiotics follow. Some of these chapters suffer from the attempt to group together compounds according to biological function, irrespective of their widely different chemical natures.

The book, in spite of its high price, can be warmly recommended, as it will be a great help to anybody who wishes to find his way quickly to up-to-date chromatographic methods.

S. E. MICHAEL

INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS. By GALEN W. EWING. Second Edition. Pp. viii + 454. New York, Toronto and London: McGraw-Hill Book Co. Inc. 1960. Price $8.90; 69s.

This book is likely to be useful to all those teachers now engaged in the teaching of instrumental methods of analysis in universities and technical colleges in this country. The level aimed at is roughly that of the undergraduate in his final year, or possibly the graduate in his first year after graduation.

The subjects covered are, to a large extent, conventional, including as they do visible, ultraviolet and infra-red absorption, X-ray diffraction, polarimetry, refractivity, electro-deposition, potentiometric, polarographic, coulometric and conductimetric methods, mass spectrometry, solvent extraction and chromatographic, ion-exchange and thermometric methods.

The opportunity has been taken in this second edition to include material on magnetic-resonance spectroscopy, gas chromatography and electrophoresis, and there is a separate chapter on electronic circuitry for analytical instruments.

Each chapter appears to follow the same general pattern, *i.e.*, the important analytical methods are first described, with sufficient theory for their general understanding, after which the most useful features of the requisite apparatus are detailed. The limitations of the various tests are discussed, and each chapter includes bibliographical notes, which, in the reviewer's opinion, have been exceedingly well selected. Some excellent problems are included, as well as useful references for further study.

Finally, descriptions are given of twenty-six practical experiments that will help the student to understand a great deal of the textual matter.

Although this book is primarily for the student, it ought to find a place on the shelves of the analytical chemist's library, and particularly of those chemists who realise how much we owe to modern physical methods of measurement in the solution of our problems.

J. HASLAM

THIOACETAMIDE AS A SULFIDE PRECIPITANT: AN ANNOTATED BIBLIOGRAPHY. Compiled by W. C. BROAD and A. J. BARNARD, jun. Pp. ii + 10. New Jersey, U.S.A.: J. T. Baker Chemical Company, March, 1960. Gratis.

The chronological arrangement of these references reflects the rapid growth of interest in this reagent. Only five out of a total of eighty-seven are dated before 1950. Two, dated 1960, bring the compilation as nearly up to date as can reasonably be expected.

Thioacetamide is not only a convenient substitute, in some circumstances, for hydrogen sulphide, but offers the advantages of precipitation in homogeneous solution, since sulphide ions are generated by hydrolysis of the reagent. Reactions with some twenty metals have been investigated in qualitative and gravimetric applications. Several papers deal with the detection of heavy metals in pharmaceutical preparations. The potentiometric titration of certain metals with thioacetamide represents another type of application.

A welcome feature of the bibliography is the inclusion, under most items, of references to *Chemical Abstracts* and *Analytical Abstracts*. These will permit the reader to satisfy the desire for fuller information that the short notes will certainly create.

This is a valuable compilation attractively presented.

W. C. JOHNSON

Publications Received

FLAME PHOTOMETRY. By JOHN A. DEAN. Pp. viii + 354. New York, Toronto and London: McGraw-Hill Book Company Inc. 1960. Price 89s.

ELEMENTARY TITRIMETRIC ANALYSIS. By A. M. G. MACDONALD, Ph.D., A.R.I.C. Pp. viii + 133. London: Butterworths Scientific Publications. 1960. Price 12s. 6d.

STYLE GUIDE FOR CHEMISTS. By LOUIS F. FIESER and MARY FIESER. Pp. vi + 116. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1960. Price $3.00; 24s.

ANNUAL REPORT 1959–60. Pp. 282. London: British Standards Institution. 1960. Price 7s. 6d.

THE RADIOCHEMISTRY OF ZIRCONIUM AND HAFNIUM. By ELLIS P. STEINBERG. Pp. vi + 52. Washington, D.C.: National Academy of Sciences—National Research Council. 1960. Price 50 cents.

Nuclear Science Series: NAS–NS–3011

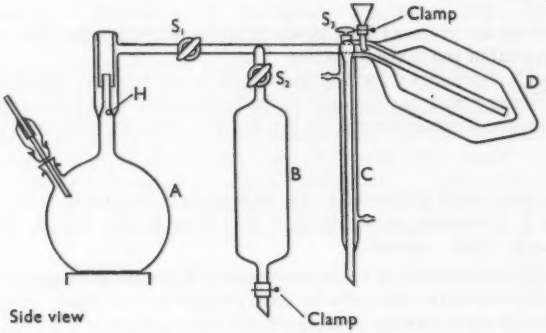
LEAD ISOTOPES IN GEOLOGY. By R. D. RUSSELL and R. M. FARQUHAR. Pp. viii + 243. New York and London: Interscience Publishers Inc. 1960. Price $9.00; 65s.

PRINCIPLES OF DAIRY SCIENCE. By ERNEST VANSTONE, D.Sc., M.Sc., F.R.I.C., and BRISTOW M. DOUGALL, M.Sc.Agric., A.R.I.C., F.G.S. Pp. 238. London: Cleaver-Hume Press Ltd. 1960. Price 25s.

FATS AND OILS: AN OUTLINE OF THEIR CHEMISTRY AND TECHNOLOGY. By H. G. KIRSCHENBAUER. Second Edition. Pp. vi + 240. New York: Reinhold Publishing Corporation; London: Chapman & Hall Ltd. 1960. Price $7.00; 56s.

Erratum

OCTOBER (1959) ISSUE, p. 619, Fig. 1. Replace lower part of figure ("Side view") by—



Ibid., p. 619, 1st line of text under Fig. 1. *For* "8 inches" *read* "6 inches."

Ibid., p. 619, 2nd line of text under Fig. 1. *For* "3 inches in diameter" *read* "45 mm outside diameter"; *for* "dimensions" *read* "outside diameter"; *omit* "wall"; *for* "2 inches greater" *read* "60 mm."

Ibid., p. 619, 3rd line of text under Fig. 1. *After* "splash-head" *insert* ", which incorporates a drain-back hole, H,".

Reprints of Review Papers

THE Society's stock of reprints of the Review Paper "The Determination of Vitamin B_{12}," by W. H. C. Shaw and Christine J. Bessell (June, 1960) has now been exhausted.

Reprints of all the other Review Papers (listed on p. 696 last month) are still available at the prices quoted.